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(54) Steroids and therapeutic compositions containing same.

(57) Steroids of the formula
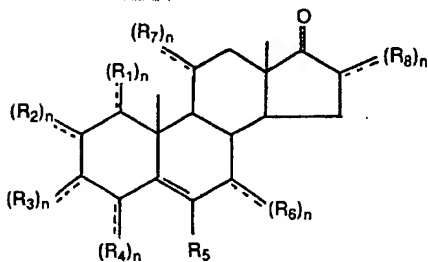
and a therapeutic composition containing same which are useful as anti-cancer, anti-obesity, anti-hyperglycemic, anti-autoimmune and anti-hypercholesterolemic agents.

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STEROIDS AND THERAPEUTIC COMPOSITIONS CONTAINING SAME

5   The invention described herein was made in
the course of work under a grant or award sponsored in
part by the National Institutes of Health.

         This invention relates to novel steroids and
more particularly to androsterone derivatives useful as
10 anti-cancer, anti-obesity, anti-diabetic and hypolipidemic
agents.

         Dehydroepiandrosterone (DHEA) and DHEA-sulfate
are major adrenal secretory products in humans. The plasma
concentration of DHEA-sulfate, which, next to cholesterol,
15 is the most abundant steroid in humans, undergoes the most
marked age-related decline of any known steroid.

         Although, DHEA-sulfate is the main precursor of
placental estrogen and may be converted into active andro-
gens in peripheral tissue, there is no obvious biological
20 role for either DHEA or DHEA-sulfate in the normal indivi-
dual. Several retrospective and prospective studies sug-
gest that women with sub-normal levels of these steroids
may be predisposed to develop breast cancer. For example,
see Brownsey et al., "Plasma dehydroepiandrosterone sul-
25 fate levels in patients with benign and malignant breast
disease," Eur. J. Cancer, 8, 131-137 (1972); Bulbrook et
al., "Relation between urinary androgen and corticoid
excretion and subsequent breast cancer," Lancet, 2, 395-
398 (1971); Rose et al. "Plasma dehydroepiandrosterone sul-
30 fate, androstenedione and cortisol, and urinary free corti-
sol excretion in breast cancer," Eur. J. Cancer, 13, 43-47
(1977); Wang et al., "Studies on the sulfate esters
of dehydroepiandrosterone and androsterone in the blood

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1 of women with breast cancer," Eur. J. Cancer, <u>10</u>, 477-482
(1974); and Zumoff et al., "Abnormal 24-hr mean plasma
concentrations of dehydroisoandrosterone and dehydroiso-
androsterone sulfate in women with primary operable breast
5 Cancer," Cancer Research, <u>41</u>, 3360-3363, September 1981.

It has also been established that DHEA is a
potent non-competitive inhibitor of mammalian glucose-
6-phosphate dehydrogenase (G6PDH). For example, see
Oertel et al. "The effects of steroids on glucose-6-
10 phosphate dehydrogenase," J. Steroid Biochem., <u>3</u>, 493-496
(1972) and Marks et al. "Inhibition of mammalian glucose-
6-phosphate dehydrogenase by steroids," Proc. Nat'l. Acad.
Sci, USA, <u>46</u>, 447-452 (1960). Moreover, Yen et al. "Pre-
vention of obesity in A^{vy}/a mice by dehydroepiandro-
15 sterone," Lipids, <u>12</u>, 409-413 (1977), reported that long-
term administration of DHEA to VY-A^{vy}/a mice prevented
the development of obesity without suppressing appetite.

Furthermore, it is also known that the long-
term treatment of C3H mice with DHEA, in addition to
20 reducing weight gain without suppressing appetite,
markedly inhibits spontaneous breast cancer development
and may delay the rate of aging. It has been observed that
DHEA antagonizes the capacity of the tumor promoter,
12-0-tetradecanoylphorbol-13-acetate, to stimulate
25 ^{3}H-thymidine incorporation in mouse epidermis and in
a cultured rat kidney epithelial cell line. See,
Schwartz, "Inhibition of spontaneous breast cancer
formation in female C3H-A^{vy}/a mice by long-term treatment
with dehydroepiandrosterone," Cancer Res., <u>39</u>, 1129-1132
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1 (1979); and Schwartz et al., "Dehydroepiandrosterone: an anti-obesity and anti-carcinogenic agent," Nut. Cancer 3, 46-53 (1981).

5 Ben-David et al., "Anti-hypercholesterolemic effect of dehydroepiandrosterone in rats," Proc. Soc. Expt. Biol. Med., 125, 1136-1140 (1967) have observed that DHEA treatment has an anti-hypercholesterolemic effect in mice, while Coleman et al. (Diabetes 31, 830, 1982) report that administration of DHEA produces a

10 marked hypoglycemic effect in C57BL/KsJ-db/db mice. The latter authors suggest that the therapeutic effect of DHEA might result from its metabolism to estrogens.

It is further known that DHEA and 16α-bromo-epiandrosterone are inhibitiors of Eptstein-Barr virus-

15 induced transformation of human lymphocytes and that 16α-bromo-epiandrosterone is a more potent inhibitor of mammalian G6PDH than DHEA. See, Schwartz et al. Carcinogensis, Vol. 2 No. 7, 683-686 (1981).

While DHEA has been found effective in the

20 afore-described manners, there is, however, evidence of an estrogenic effect after prolonged administration. DHEA is not an estrogen per se but is well known to be con-vertible into estrogens. In addition, the therapeutic dose of DHEA is rather high. It would therefore be

25 highly desirable to provide steroids, which while having the same afore-described advantages of DHEA are more potent and do not produce an estrogenic effect.

Accordingly, the present invention provides novel steroids.

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1   The steroids of the present invention
exhibit significant and desirable pharmalogical
properties, and are particularly useful as cancer
preventive agents.

5   These steroids are additionally useful
as anti-obesity agents, anti-hyperglycemic agents,
anti-aging agents, and anti-hypercholesterolemic
agents.

This invention further provides steroids
10 useful as anti-cancer, anti-obesity, anti-hyper-
glycemic, anti-aging and anti-hypercholesterolemic
agents, which do not evidence estrogenic effects.

The present invention also provides a
process for the treatment and/or prevention of cancer,
15 obesity, aging, diabetes, and hyperlipidemia.

The present invention provides novel steroids
of the general formula:
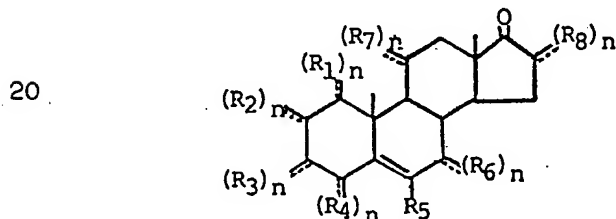
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wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are each inde-
25 pendently selected from the group consisting of hydrogen,
alkyl, alkenyl, alkynyl, halogen and hydroxyl, n is an
integer from 1 to 2 inclusive with the proviso that
when R_1, R_2, R_3, R_4, R_6, R_7 or R_8 are alkenyl or
alkynyl, n is 1; with the further provisos that when

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1 R_3 is hydroxy, any one of the substituents R_1, R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_1 may only be alkyl when any one of R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is
5 hydroxy, R_2 may only be hydroxy when any one of R_1, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_4 may only be halogen with R_1, R_2, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_6 may only be hydroxy or methyl when R_1, R_2, R_4, R_5,
10 R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_7 may only be hydroxy when R_1, R_2, R_4, R_5, R_6 or R_8 is other than hydrogen; and when R_3 is hydroxy, R_8 may only be methyl, ethyl, hydroxy or halogen when, R_1, R_2, R_4, R_5, R_6 or R_7 is other than hydrogen; and
15 when R_3 is hydroxy, R_5 may be alkyl only when R_1, R_2, R_4, R_6, R_7 or R_8 are other than hydrogen. Preferably, R_3 is alkyl of from 1 to 10 carbon atoms and most preferably lower alkyl of from 1 to 5 carbon atoms.
The present invention further provides
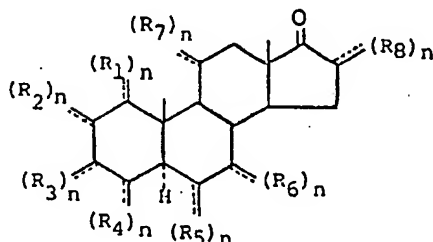20 novel steroids of the formula:
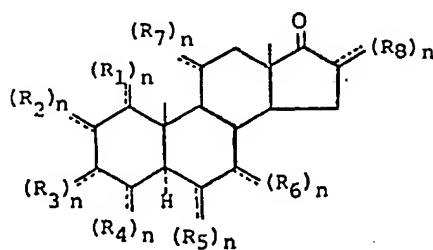
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wherein R_1-R_8 are selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halogen and hydroxyl, n is an integer from 1 to 2 inclusive with the proviso that when R_1-R_8 are alkenyl or alkynyl n is 1 and with the further provisos that R_3 may be hydroxy or halogen only when any one of R_1, R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_1 may be hydroxy or halogen only when any one of R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_2 may be methyl or halogen only when any one of R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_4 may be halogen, methyl or hydroxy only when any one of R_1, R_2, R_3, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_5 may be methyl, halogen or hydroxy only when R_1, R_2, R_4, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_6 may be hydroxy or methyl only when R_1, R_2, R_4, R_5, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_7 may be hydroxy only when R_1, R_2, R_4, R_5, R_6 or R_8 is other than hydrogen; and when R_3 is hydroxy, R_8 may be methyl, hydroxy or halogen only when R_1, R_2, R_4, R_5, R_6 or R_7 is other than hydrogen.

The present invention additionally provides processes for the prophylaxis of cancer, obesity, aging, diabetes and hyperlipidemia by administering to a host, e.g. mammals, a therapeutically effective amount of the afore-identified steroids.

The present invention further provides processes for the prophylaxis of cancer, obesity, aging, diabetes, hyperlipidemia comprising administering to

1 a host, e.g. mammals, a therapeutically effective amount of
the afore-identified steroids or a steroid having the general
formula:

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$$(R_7)_n \quad O \quad (R_8)_n$$
$$(R_1)_n$$
$$(R_2)_n$$
$$(R_3)_n \quad H \quad (R_6)_n$$
$$(R_4)_n \quad (R_5)_n$$

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wherein R_1-R_8 are selected from the group consisting of
hydrogen, alkyl, alkenyl, alkynyl, halogen and hydroxyl,
15 n is an integer from 1 to 2 inclusive and with the pro-
viso that when R_1-R_8 are alkenyl or alkynyl n is 1.

In accordance with the present invention, it
has been surprisingly discovered that steroids having a
certain structure, described hereinafter in more detail,
20 are characterized with significant pharmacological pro-
perties without toxic or undesirable estrogenic effects.
That is, it has been quite unexpectedly discovered that
the steroids of the present invention are useful as can-
cer preventive, anti-obesity, anti-diabetic, anti-aging
25 and anti-hypercholesterolemic agents, but unlike DHEA are
more potent and exhibit very little or no estrogenic
effects.

More particularly, the steroids of the present
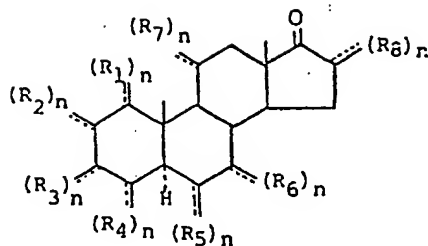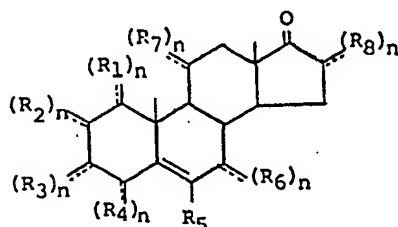invention have the general formulas:

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and

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15 wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are as defined
hereinbefore. The R_1-R_8 substituents are designated as
being in the α-position by means of a broken line (---)
joining the substituent to the steroid nucleus, the sub-
stituents are designated as being in the β-position by
20 means of a solid line (——) joining the substituent to
the steroid nucleus and in those cases in which the sub-
stituent may be either in the α- or β- position the sub-
stituents are indicated as being joined to the steroid
nucleus by a broken line and a solid line placed side to
25 side. Furthermore, in accordance with I.U.P.A.C. nomen-
clature, the carbon atoms of the steroids of the present
invention are numbered as follows and the steroids have
the designated I.U.P.A.C. stereochemistry:

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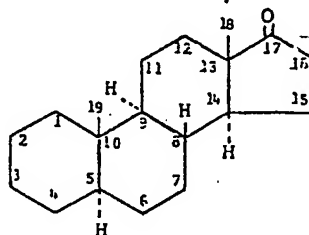
1   Specific illustrative compounds within the
above structural formulas and useful in accordance with
the present invention include:

     3β-hydroxy-1α-methylandrost-5-en-17-one.

5   1α-methylandrost-5-en-17-one

     1α-methyl-5α-androstan-17-one

     3β-hydroxy-2α-methylandrost-5-en-17-one

     2α-ethynyl-3β-hydroxyandrost-5-en-17-one

     3β-hydroxy-2α,6-dimethylandrost-5-en-17-one

     2α,6,16α-trimethylandrost-5-en-17-one

10   3β-hydroxy-2α,6,16α-trimethylandrost-5-en-17-one

     3β-hydroxy-2α-ethynyl-6,16α-dimethylandrost-5-en-17-one

     2α-ethynyl-6-chloroandrost-5-en-17-one

     3β-methylandrost-5-en-17-one

     3β-ethenylandrost-5-en-17-one

15   3β-ethynylandrost-5-en-17-one

     3β-ethynyl-6-methylandrost-5-en-17-one

     3β-ethynyl-6-chloroandrost-5-en-17-one

     3β-ethynyl-6-chloro-16α-methylandrost-5-en-17-one

     3β-ethylandrost-5-en-17-one

20   3β-butylandrost-5-en-17-one

     3β-ethynyl-6,16α-dimethylandrost-5-en-17-one

     3β.16α-diethynylandrost-5-en-17-one

     3β-ethynyl-6-methyl-16α-ethylandrost-5-en-17-one

     3β-ethynyl-7β-methylandrost-5-en-17-one

25   2α,7β-dimethylandrost-5-en-17-one

     1α-chloro-3β-methylandrost-5-en-17-one

     3β-hydroxy-4α-methylandrost-5-en-17-one

     3β-hydroxy-4α-ethynylandrost-5-en-17-one

30   3β-hydroxy-4α-ethenylandrost-5-en-17-one

     3β-hydroxy-16α-ethyl-4α-ethynylandrost-5-en-17-one

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1   3β-hydroxy-16α-methyl-4α-ethynylandrost-5-en-17-one

    2α,3β-dihydroxyandrost-5-en-17-one

    2α,3β-diethynylandrost-5-en-17-one

    3β-hydroxy-4,6-dimethylandrost-5-en-17-one

5   3β-methyl-4α-ethynylandrost-5-en-17-one

    3β-methyl-7β-chloroandrost-5-en-17-one

    3β-methyl-16α-ethylandrost-5-en-17-one

    3β-methyl-16α-ethynylandrost-5-en-17-one

    3β-hydroxy-6-ethylandrost-5-en-17-one

10 3β-hydroxy-11α-methylandrost-5-en-17-one

    3β-hydroxy-11α-chloroandrost-5-en-17-one

    3β-hydroxy-16α-methylandrost-5-en-17-one

    3β-hydroxy-16α-ethylandrost-5-en-17-one

    3β-hydroxy-16α-ethenylandrost-5-en-17-one

15 3β-hydroxy-16α-ethynylandrost-5-en-17-one

    3β-hydroxy-6-ethenylandrost-5-en-17-one

    3β-hydroxy-6-ethynylandrost-5-en-17-one

    2α-methyl-3β-hydroxy-6-ethynylandrost-5-en-17-one

    3β-hydroxy-7β-methylandrost-5-en-17-one

20 3β-hydroxy-7β-ethenylandrost-5-en-17-one

    3β-hydroxy-7β-ethynylandrost-5-en-17-one

    2α-methyl-3β-hydroxy-7β-ethynylandrost-5-en-17-one

    2α,3β-dimethylandrost-5-en-17-one

    3β,4α-dimethylandrost-5-en-17-one

25 2α,3β-diethynylandrost-5-en-17-one

    3β,4α-diethynylandrost-5-en-17-one

    2α,3β-diethenylandrost-5-en-17-one

    3β,4α-diethenylandrost-5-en-17-one

    2α,3β,6-trimethylandrost-5-en-17-one

30 3β,4α,7βb-trimethylandrost-5-en-17-one

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3β-ethynyl-7β-methylandrost-5-en-17-one

6-methylandrost-5-en-17-one

7β-methylandrost-5-en-17-one

11α-methylandrost-5-en-17-one

16α-methylandrost-5-en-17-one

3β-hydroxy-4,4-difluoroandrost-5-en-17-one

3β-hydroxy-16,16-difluoroandrost-5-en-17-one

2α-fluoro-3β-hydroxyandrost-5-en-17-one

3β-hydroxy-6-bromoandrost-5-en-17-one

3β-methyl-5α-androstan-17-one

3β-methyl-4α-ethynyl-5α-androstan-17-one

3β-methyl-7β-chloro-5α-androstan-17-one

3β-methyl-16α-ethyl-5α-androstan-17-one

3β-methyl-16α-ethynyl-5α-androstan-17-one

3β-hydroxy-6-ethyl-5α-androstan-17-one

3β-hydroxy-11α-methyl-5α-androstan-17-one

3β-hydroxy-11α-chloro-5α-androstan-17-one

3β-hydroxy-16α-methyl-5α-androstant-17-one

3β-hydroxy-16α-ethyl-5α-androstant-17-one

3β-hydroxy-16α-ethynyl-5α-androstan-17-one

3β-hydroxy-6-ethenyl-5α-androstan-17-one

2α-methyl-3β-hydroxy-6-ethynyl-5α-androstan-17-one

3β-hydroxy-7β-methyl-5α-androstan-17-one

3β-hydroxy-7β-ethenyl-5α-androstan-17-one

3β-hydroxy-7β-ethynyl-5α-androstan-17-one'

2α-methyl-3β-hydroxy-7β-ethynyl-5α-androstan-17-one

2α,3β-dimethyl-5α-androstan-17-one

3β,4α-dimethyl-5α-androstan-17-one

2α,3β-diethynyl-5α-androstan-17-one

3β,4α-diethynyl-5α-androstan-17-one

2α,3β-diethenyl-5α-androstan-17-one

16α-bromo-3β-methylandrost-5-en-17-one

1 The steroids of the present invention may be prepared in accordance with conventional organic synthe- ses or if known may be commercially obtained. The fol- lowing procedures are illustrative of some procedures
5 which may be utilized to prepare the steroids included herein:

A representative procedure for alkylation at carbon-1 and specifically the synthesis of a 1α-methyl DHEA **3a** and 1α-methyl-desoxy DHEA **3b** is given in
10 Scheme 1.

Scheme 1

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1.Na₂CO₃/MeOH
2.CrO₃/pyr

reduction

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10 a   **3b** R=2H
     c R=O

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3a

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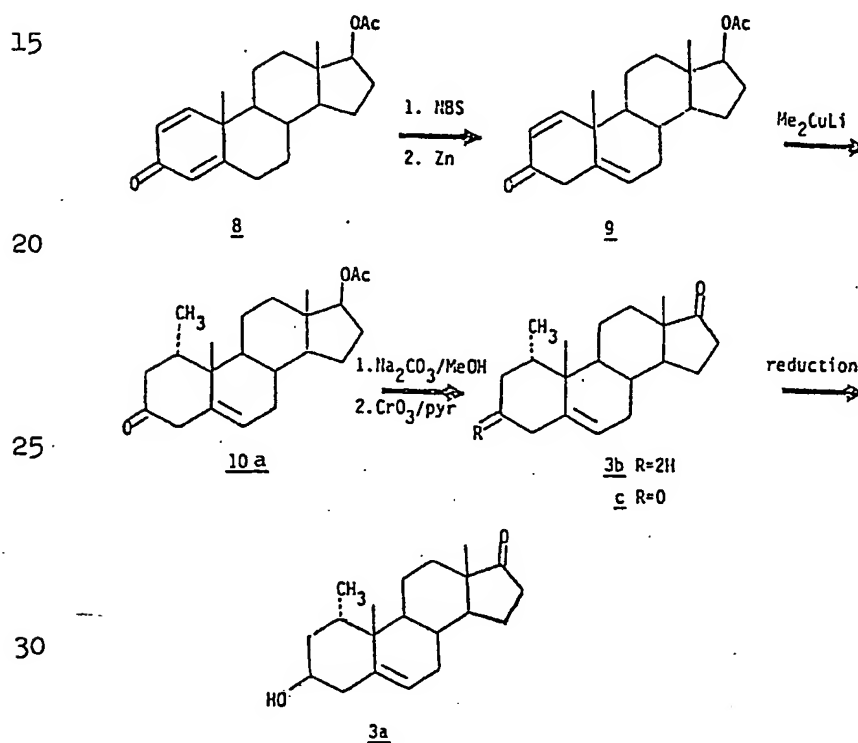
1   Allylic bromination (e.g. with N-bromosuccini-
mide (NBS)) of 17β-acetoxyandrosta-1,4-dien-3-one **8** followed
by treatment with zinc affords the non-conjugated enone **9**.
1,4-Alkylation with lithiodimethyl cuprate provides the
5 1α-methyl ketone **10a**. At this stage the **10a** may be con-
verted to a methylene by Wolff-Kishner reduction. These
vigorous reaction conditions result in hydrolysis of the
resulting carbon-17 acetate thereby yielding the hydroxy
desoxy derivative, 17β-hydroxy-1α-methylandrost-5-ene (**3b**)
10 Both **10a** and its desoxy derivative can be converted via
standard reactions i.e. hydrolysis of the 17-acetate with
sodium carbonate and methanol followed by chromium tri-
oxide oxidation of the resulting 17-alcohol to the carbon-17
ketone. Selective reduction of the carbon-3 ketone, 3,17-
15 diketone **3c** using sodium borohydride pyridine (pyr) yields
1α-methyl dehydroepiandrosterone **3a**.

     The following procedures are illustrative for
alkylation at carbon-2 and are figuratively illustrated
in scheme 2 below.
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Methylation of testosterone (1) using lithium
diisopropylamide (LDA) and methyl iodide afforded a mixture
of 2α- and 2β-methyl-17β-hydroxy-4-androsten-3-one (2 & 3).
5. Treatment of the mixture with sodium methoxide in methanol
epimerizes the 2β-axial methyl to the 2α-configuration (2).
Acetylation of 2 with acetic anhydride (Ac₂O) and p-toluene sul-
fonic acid (PTSA) in toluene afforded 2α-methyl-3,17β-dihydroxy-
3,5-androstadien-3,17-diacetate (4). Treatment of the
10 diacetate (4) with sodium borohydride in 95% ethanol yielded
2α-methyl-3β,17β-dihydroxy-5-androsten-17-acetate (5).
Protection of the 3-hydroxy group as a tetrahydropyranyl
ether followed by hydrolysis of the 17-acetate yielded
2α-methyl-3β,17β-dihydroxy-5-androsten-3-tetrahydropyranyl
15 ether 7. Oxidation of the C-17 hydroxy group in 7 followed
by hydrolysis of the tetrahydropyranyl ether with hydro-
chloric acid and aqueous acetone yielded 3β-hydroxy-2α-
methylandrost-5-en-17-one (9).

The following is a specific example for the
20 synthesis of 2α-methyl DHEA.

To a solution of diisopropylamine (5.3 ml, 38 mmol)
in freshly distilled tetrahydrofuran (80 ml) stirred at
-78°C was added n-butyllithium (29.3 ml of 1.3 M in hexane,
38 mmol). This was stirred at -78°C for 30 minutes then
25 warmed to -30° and 17β-hydroxy-4-androsten-3-one (1)
(5.0 g, 17.3 mmol) in tetrahydrofuran (30 ml) was added
dropwise. After 30 minutes at -30°C iodomethane (4 ml.
80 mmol) was added. The mixture was allowed to slowly
warm to room temperature with stirring, then saturated
30 ammonium chloride solution was added and the product was
extracted with ether. The organic layer was dried and
the solvent removed to give a mixture of isomers 2 & 3
as an oil (5.26 g) which was used in the next step.

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1   To a stirred solution of sodium (0.75 g, 32 mmol)
dissolved in methanol (100 ml) was added the epimeric mix-
ture of 2-methyl-17β-hydroxy-4-androsten-3-one, 2 & 3
(4.93 g, 16.2 mmol) in methanol (100 ml). After 17 hours
5 at room temperature, saturated ammonium chloride solution
was added and most of the solvent was removed _in vacuo._
The product was extracted with dichloromethane, washed
with water, dried and the solvent removed to give a gum
(4.86 g) which was purified by column chromatography on
10 silica gel. Elution with hexane ether gave 1.6 g of 2
which crystallized from methanol mp 149-151°C;

H^1 NMR (CDCl$_3$) δ5.64 (s, 1, H-4), 3.60 (t, 1, H-17, J=9Hz),
1.24 (s, 3, H-19), 1.13 (d, 3, H-2 methyl, J=6 Hz), 0.83
(s, 3, H-18); MS m/e 302(M^+,33), 260(21), 246(29), 28(100).
15

       A solution of 2α-methyl-17β-hydroxy-4-andro-
sten-3-one (2) (4.86 g, 16.1 mmol) product mixture from
previous step in acetic anhydride (40 ml) and paratoluene
20 sulfonic acid (200 mg) in toluene (100 ml) was refluxed
3 1/2 hours. Pyridine (1 ml) was added and the mixture
was concentrated on a rotary evaporator then partitioned
between methylene chloride and water. The organic layer
was dried and the solvent removed. The product mixture
25 (5.78 g) was separated on a flash silica column to give
2α-methyl-3,17β-dihydroxy-3,5-androstadien-3,17-diacetate
(4) 1.81 g (27.4%) crystallized from Et$_2$0 - hexane mp
170-171°C.

30 H^1 NMR(CDCl$_3$) δ 5.57 (s, 1, H-4), 5.40 (m, 1, H-6), 4.55
(t, 1, H-17, J=9Hz), 2,08 (s, 3, 3-acetate), 2.01 (s, 3,
17-acetate), 1.06 (s, 3, H-19), 0.98 (d, 3, 2 methyl,
J=6 Hz), 0,83 (s, 3, H-18); MS m/e 386(M^+,3) 358(12),
43(100).
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1   A suspension of 2α-methyl-3,17β-dihydroxy-
3,5-androstadien-3,17-diacetate (4) (1.31 g, 3.4 mmol)
and sodium borohydride (1.3 g) in 95% ethanol (100 ml)
was stirred at room·temperature for 3 1/2 hours. The

5 solution was cooled to 0°C and glacial acetic acid was
added, followed by saturated sodium bicarbonate solution.
The product was partitioned between dichloromethane and
water, the organic layer dried, and the solvent removed
to give 1.23 g product mixture which was separated on

10 40 g of flash silica column eluted to give 5, 0.7 g
from ether hexane) mp 179-182°C;

H[1] NMR (CDCl$_3$)δ 5.27 (m, 1, H-6), 4.62 (t, 1, H-17, J=9Hz),
3.03 (t, 1, H-3, J=9Hz) 2.05 (s, 3, 17-acetate), 1.07
(s, 3, H-19), 1.02 (d, 3, 2-methyl, J=8Hz), 0.83 (s, 3,
15 H-18).

          A solution of 2α-methyl-3β,17β-dihydroxy-5-
androsten-17-acetate 5 (1.42 g, 4.1 mmol) dihydropyran (DHP)
(10 ml) and paratoluene sulfonic acid (100 mg) in ether
(50 ml) was stirred at room temperature for 17 hours.
20 The ether solution was washed with saturated sodium
bicarbonate solution then water, dried and solvent
removed to give the product mixture as an oil (1.65 g)
The product was not purified but carried on to the next
step.
25   2α-Methyl-3β,17β-dihydroxy-androst-5-ene-3-tetra-
hydropyranyl ether 17-acetate, 6, from the previous step
(1.65 g, 3.84 mmol) was dissolved in a solution of 5%
potassium carbonate in 4:1 methanol:water (100 ml) and
refluxed 1.5 hours. Most of the solvent was removed
30 under reduced pressure and the product was partitioned
between chloroform and water. The organic layer was dried
and solvent removed to give 1.45 g of the product 7
which was used on the next step.

35

1   The product mixture $\underline{7}$ from previous step
   (1.45 g, 3.84 mmol) was dissolved in pyridine (10 ml)
   and added to the complex formed by mixing chromium
   trioxide (2 g) in pyridine (20 ml). This was stirred
5 2 1/2 hours at room temperature then 1:1 ether:benzene
   (30 ml) was added and the mixture was filtered through
   celite then silica gel. The solvent was removed to
   give the product mixture $\underline{8}$, 1.52 g as an oil which
   was carried on to the next step.

10   A solution consisting of the product mixture $\underline{8}$
   from the previous step (1.52 g, 3.94 mmol) and 3N HCl
   (2 ml) in acetone (40 ml) was stirred at room tempera-
   ture for 3 hours. Saturated sodium bicarbonate solution
   was added and the product was extracted with dichloro-
15 methane. The organic layer was dried and the solvent
   removed to give 1.17 g product mixture which was separted
   on a flash silica column. Elution with 30:70 ether:hexane
   gave 3β-hydroxy-2α-methyl-androst-5-en-17-one ($\underline{9}$)(317 g)
   which was crystallized from ether:hexane mp 171.5-173;
20 H^1 NMR(CDCl$_3$)δ 5.45 (m, 1, H-6), 3.10 (broad m, 1, H-3)
   1.13 (s, 3, H-19), 1.07 (d, 3, 2 methyl, J=8Hz), 0.97
   (s, 3 H-18).

           As stated before, the above reactions involving
   alkylation at carbon-2 are figuratively illustrated in
25 Scheme $\underline{2}$.

30

35

Scheme 2

1. LDA

2. CH₃I

$\xrightarrow{\text{1. LDA}}$

$\xrightarrow{\text{CH}_3\text{ONa}}_{\text{CH}_3\text{OH}}$

1   **2 & 3**

$\xrightarrow[\text{C}_6\text{H}_5\text{CH}_3]{\text{Ac}_2\text{O}}_{\text{pTSA}}$

$\xrightarrow[\text{95\% EtOH}]{\text{NaBH}_4}$

2   **4**

$\xrightarrow[\text{Et}_2\text{O}]{\text{DHP}}_{\text{pTSA}}$

$\xrightarrow[\text{pyridine}]{\text{CrO}_3}$

5   **6**, R=COCH₃
                         7, R=H

$\xrightarrow[\text{acetone}]{\text{HCl}}_{\text{H}_2\text{O}}$

8   **9**
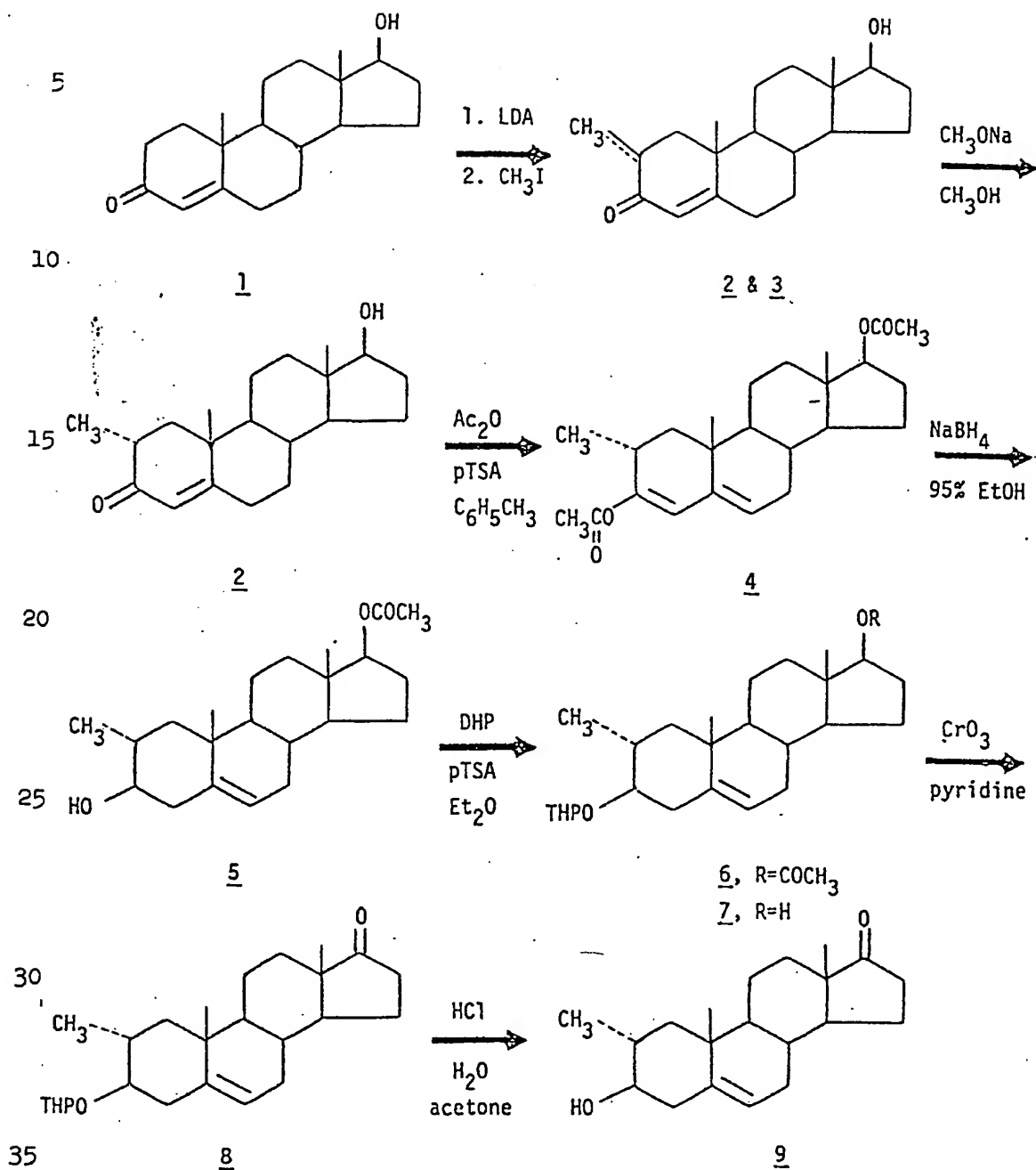
1   The following procedures are representative
for carbon-3 alkylations, shown figuratively in scheme 3 below.
        Synthesis of dehydroepiandrosterone with a
methyl group replacing the hydroxyl group at carbon-3
5 is shown below in scheme 3. The methyl configuration
at carbon-3 is β, as determined by X-ray analysis.
3β-Hydroxyandrost-5-en-17-one   (10) was iodonated
at carbon-3 with catechol phosphochloridate followed
by iodine. 3β-Iodoandrost-5-en-17-one (11) was
10 ketalized then alkylated with a mixture of methyl
lithium and cuprous cyanide, in tetrahydrofuran to
yield 3β-methylandrost-5-en-17-ethylene ketal (13).
Hydrolysis of the ketal afforded 3β-methylandrost-5-
en-17-one (14).

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Scheme 3

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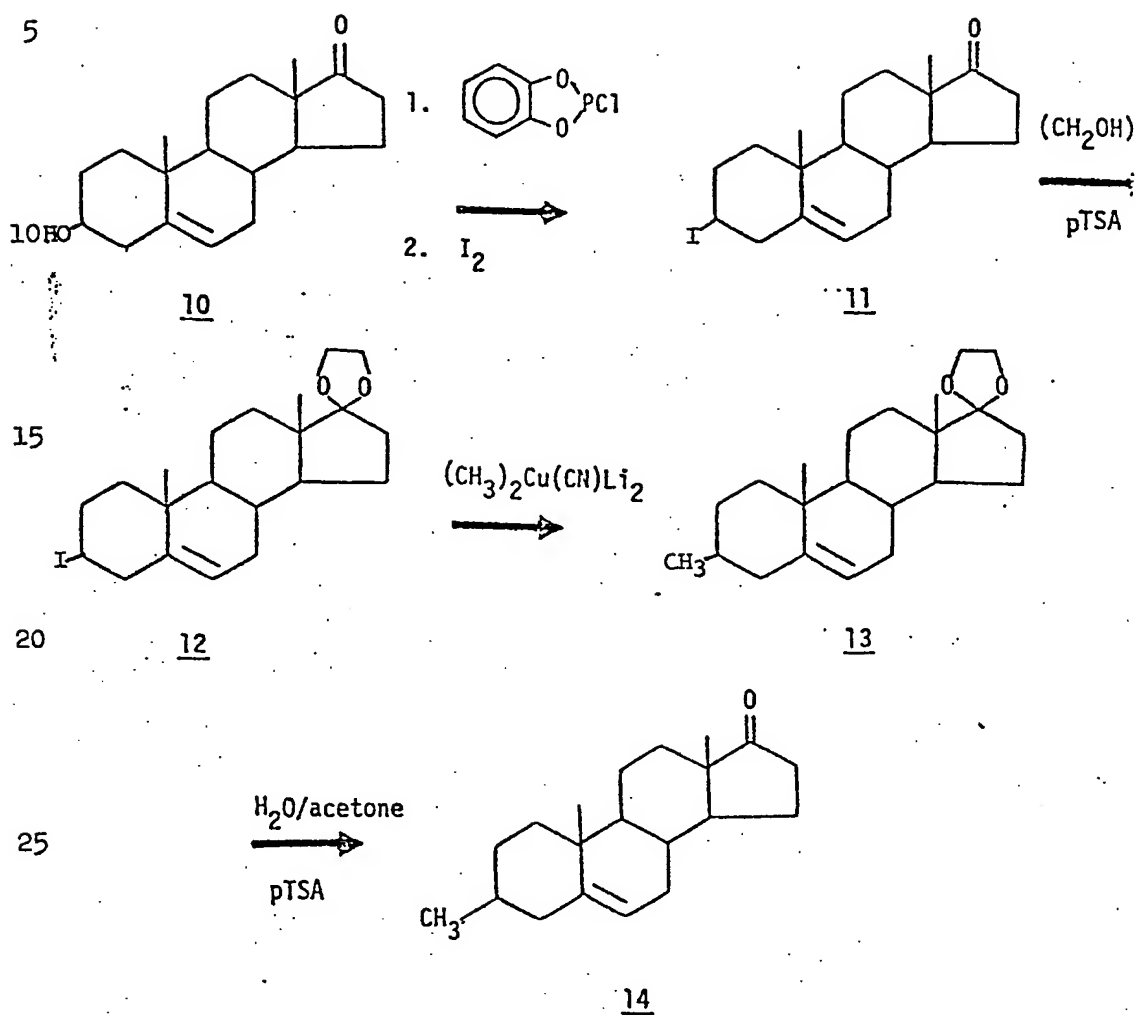
1   More specifically, 3β-iodoandrost-5-en-17-one
(11) (11.83g, 29.7 mmol) ethylene glycol (20 ml) and
p-toluene sulfonic acid (200 mg) in benzene (250 ml)
were refluxed under a Dean-Stark trap for 72 hrs. The
5 solution was washed with saturated sodium bicarbonate,
water, then dried over magnesium sulfate. Evaporation
and recrystallization from ether afforded 11.5g (87.3%)
of 3β-iodoandrost-5-en-17-one 17-ethyleneketal (12):
mp 140-141°C, IR(KBr) 3010, 2940, 1470, 1425, 1375 cm^{-1}
10 ^{1}H NMR (CDCl$_3$)δ 5.44 (brd J=6Hz, 1H, H-6) 3.91 (s, 4H,
ketal) 1.07 (s, 3H, C-19 Me) .88 (s, 3H, C-18 Me);
MS (m/e) 442 (M$^+$, 1), 380 (35), 315 (57), 253 (67),
227 (11), 105 (24), 99 (100), 91 (35), 55 (27), 41 (33).

    Cuprous cyanide (4.465 g, 49.9 mmol) was placed
15 in a dry 500 ml 3 neck round bottom flask equipped with a
magnetic stirrer. The system was flushed with N$_2$ and dry
THF (30 ml) was added. The suspension was cooled to -78°C
and MeLi 1.5 M (66.5 ml, 99.8 mmol) was added via syringe.
The solution was allowed to warm to 0°C for 5 min., which
20 resulted in a clear tan solution.

    After recooling to -78°C, the 3β-iodo-17-ketal
(3) (7.35 g 16.6 mmol) in 40 ml dry tetrahydrofuran was
added via a syringe and the solution allowed to warm to
room temperature and stirred for 18 hrs. under N$_2$. The
25 solution was extracted with 100 ml of 90% saturated
NH$_4$Cl/10% conc. NH$_4$OH. The organic layer was separated,
dried over MgSO$_4$ and evaporated to give 6.69 g of crude
product. Chromatography on flash silica (240 g) and elu-
tion with 1% Et$_2$0/99% hexane gave 6.41 g of colorless
30 crystals. Recrystallization from methanol (200 ml) gave
3β-methylandrost-5-en-17-one 17-ethyleneketal (4).

35

1 mp 121-122°C Anal. Calc. C 80.06 H 10.38.

Found C 80.12 H 10.55

IR(KBr) 3010, 2930, 1450, 1430, 1370; ^{1}H NMR (CDCl$_3$)δ

5.33 (brd J=6Hz, 1H, H-6) 3.90 (s, 4H, ketal) 1.03

5 (s, 3H, C-19 Me) .91 (s, 3H, C-18 Me) .97 (d, 3H, C-3

Me); MS (m/e) 330 (M$^+$, 16), 316(7), 268(29), 253(22),

239(9), 99 (100), 91(22), 55(27), 41(22).

The 3β-methylandrost-5-en-17-one 17-ethylene-

ketal (<u>13</u>) (2.20 g 6.7 mmol) was dissolved in acetone

10 (100 ml). p-Toluene sulfonic acid (100 mg) and H$_2$O

(20 ml) were added and the solution refluxed for 2 hrs.

The solution was evaporated, taken up in ether (30 ml),

washed with saturated NaHCO$_3$, H$_2$O, then dried over MgSO$_4$.

The solution was filtered and evaporated to give a color-

15 less solid which was recrystallized from methanol to

give 3β-methylandrost-5-en-17-one (<u>14</u>) colorless plates

1.17 g (61%).

mp 148-150°C; IR(KBr) 3010, 2910, 1740, 1455, 1430, 1365;

H^{1}NMR (CDCl$_3$) δ5.41 (brd, J=6Hz, 1H, H-6) 1.11 (s, 3H,

20 C-19 Me) 0.99 (s, 3H, C-18 Me) 1.07 (d, 3H, C-3 Me); MS

(m/e) 286 (M$^+$, 58) 271(51), 229 (31), 159 (36), 105 (72),

91 (95), 79 (89), 55 (9), 41 (100).

Anal. Calc. C 83.85 H10.55

Found C 83.66 H10.65

25   Androst-5-en-17-one <u>15</u> (Desoxy DHEA)
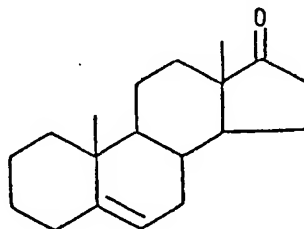
30

<u>15</u>

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1 Androst-5-en-17-one (<u>15</u>) mp 106° is synthesized in
   accordance with T. Nambara and H. Takahaski, <u>Chem.</u>
   <u>Pharm</u>. <u>Bull</u>. <u>Jap</u>., 1970, <u>18</u>, 2309 m.p. 108-109°C.

5   A procedure for carbon-4 alkylation and the
   synthesis of 4α-methyl DHEA is given in Scheme 2.

<p style="text-align:center"><u>Scheme 4</u></p>

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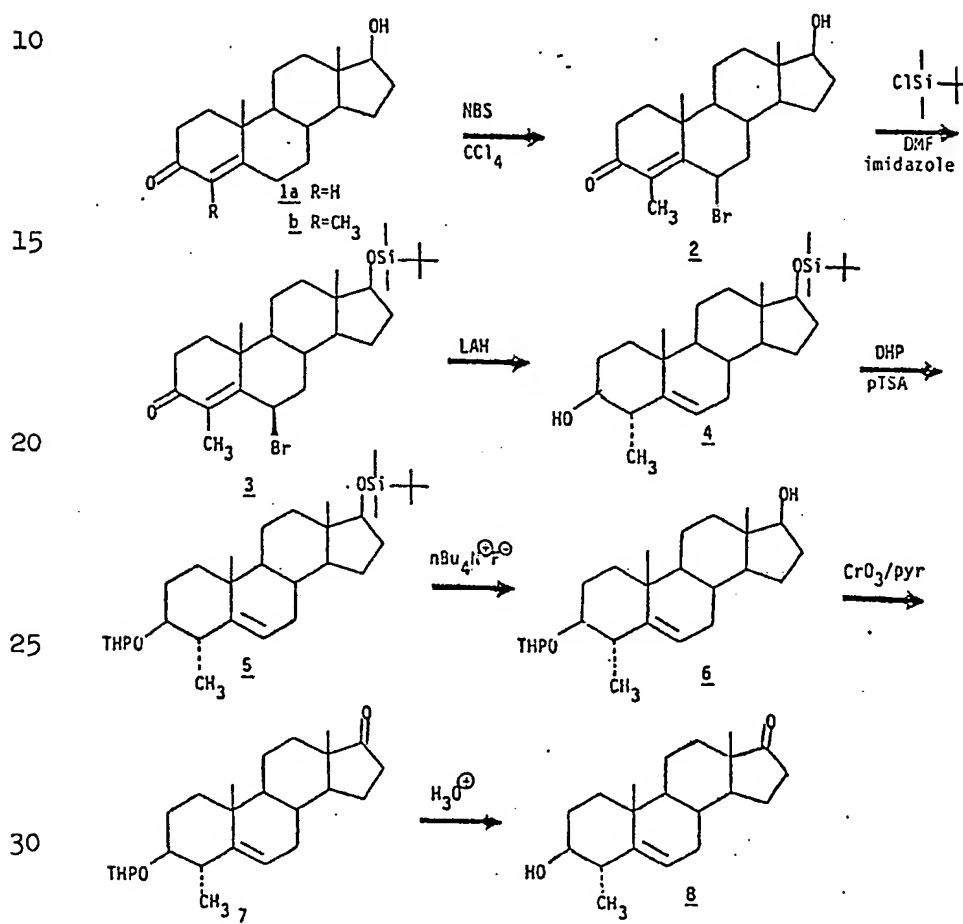
With reference to Scheme 4, alkylation of testerone
1a using potassium t-butoxide and methyl iodide according
to the method of Atwater yielded 4-methyltestosterone 1b.
Allylic bromination of 4-methyltestosterone using N-bromo-
5   succinimide in carbon tetrachloride yields the 6ß-bromo-
4-methylandrost-4-en-17ß-ol-3-one 2.Protection of the C-17
alcohol as its t-butyldimethyl silyl derivative yields 3.
Lithium aluminum hydride reduction of the ketone in 3 with
concomitant double bond migration and loss of bromide
10   should yield 4. Protection of the C-3 alcohol as a tetra-
hydropyranyl ether, followed by deprotection and oxidation
of the C-17 alcohol should yield the C-17 ketone 7. Removal
of the C-3 tetrahydropyranyl ether should yield 4 α-methyl
dehydroepiandrosterone 8.

15

ALKENYLATION AND ALKYLATION AT CARBON-6

Steroids may be alkylated at carbon-6 using the
method of U. Stache and W. Fritsch Liebigs Analen 1966,
697, 204.

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$Ph_3P^+-C^-HR$
Wittig

H_2O
pTSA

25   1   2

30   3
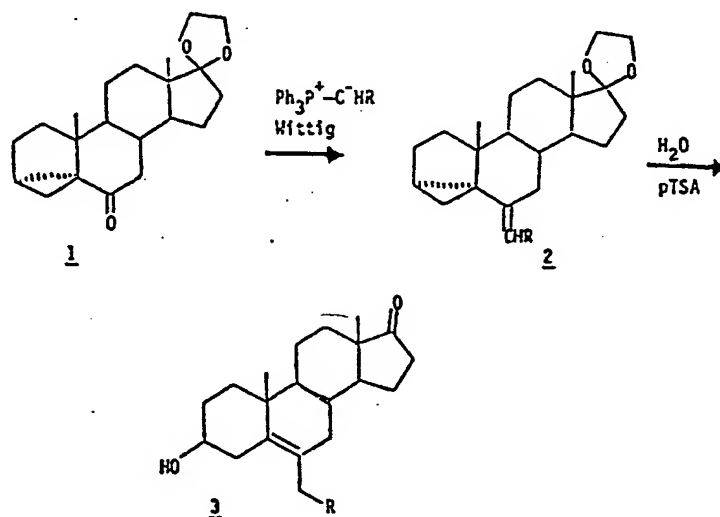
1   3α,5-Cyclosteroids such as 3α,5-cyclo-5α-androstan-6,17-dione 17 ketal <u>1</u> are readily available by solvolysis of steroidal 5-ene-3β-tosylates and mesylates followed by oxidation of the C-6 hydroxyl group,

5 Methylenation of <u>1</u> affords 6-methylene-3α,5-cyclo-5α-androstan-17-one 17-ketal <u>2</u> (R=H). Treatment of <u>2</u> with aqueous acid results in the addition of water and the formation of 3β-hydroxy-6-methylandrost-5-en-17-one, <u>3</u> (R=H). Alkenylated derivatives of <u>3</u> may be synthe-

10 sized starting with the appropriated substituted Wittig reagent, such as $Ph_3P^{\oplus}$—— $\overset{\ominus}{CH}$—CH=CH$_2$.

<u>Alkylation at C-7</u>

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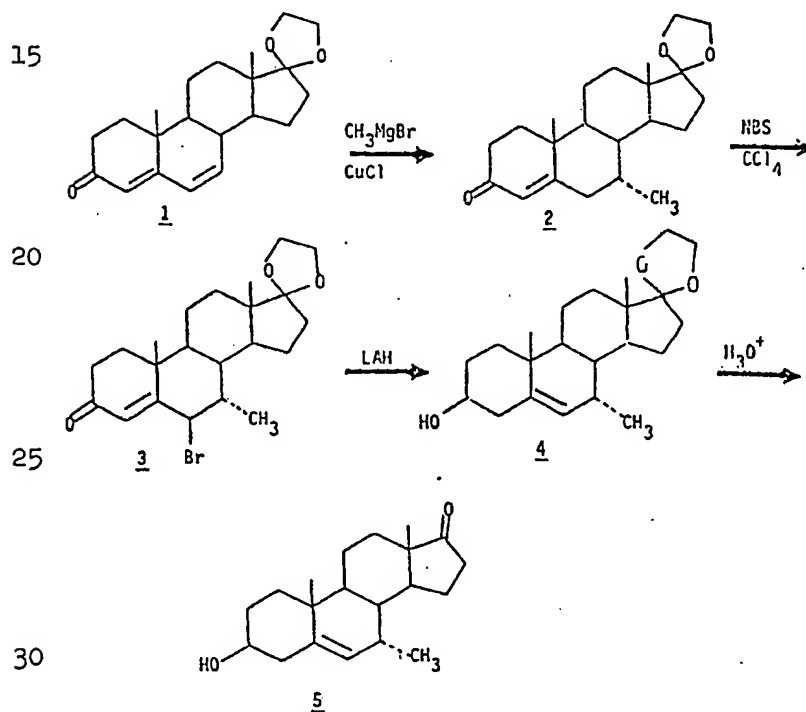
1   Alkylation of androsta-4,6-dien-3,17-dione 17
ketal $\underline{1}$ with methyl magnesium bromide in the presence of
cuprous chloride, proceeds via conjugate addition to
yield 7α-methylandrost-5-en-3,17-dione 17 ketal $\underline{2}$. Ally-

5 lic bromination of $\underline{2}$ using N-bromosuccinimide in carbon
tetrachloride yields the 6β-bromo-7α-methylandrost-4-en-
3,17-dione 17 ketal $\underline{3}$. Lithium aluminum hydride reduc-
tion of the ketone in $\underline{3}$ with concomitant double bond
migration and loss of bromide should yield $\underline{4}$. Depro-

10 tection of the C-17 ketone with aqueous acid yields
3β-hydroxy-7α-methylandrost-5-en-17-one, $\underline{5}$. Higher
homologues may be synthesized using the substituted
Grignard reagent i.e. R=CH_3, C_2H_5, C_3H_7. The 7β-epimer
can be synthesized by treatment of $\underline{2}$ with DDQ--dichloro-

15 dicyanoquinone to generate another olefin at C-7. Cata-
lytic reduction of this olefin should occur from the
α face of the steroid to yield the 7β-methyl steroid
i.e. 7β-methylandrost-5-en-3,17-dione 17 ketal. Follow-
ing the same sequence as above yields 3β-hydroxy-7β-

20 methylandrost-5-en-17-one.

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1 Alkylation at Carbon-11
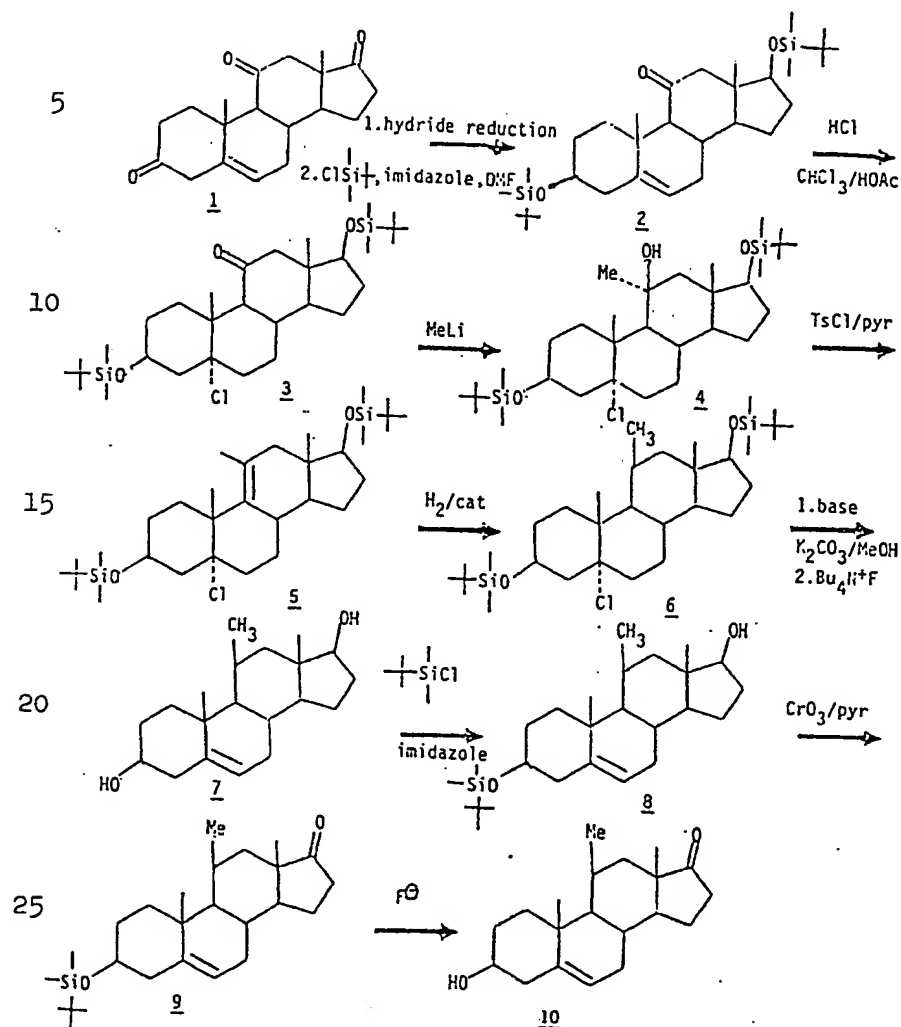
1   Due to the hindered nature of the C-11
ketone, selective reduction of androst-5-en-3,11,17-
trione 1 with hydride should yield the C-3, C-17
dihydroxy steroid 2a, R=H which is protected as its
5 bis(dimethyl-tert-butylsilyl)ether 2b R=Si(CH₃)₂t-Bu. Addition
of hydrogen chloride across the C-5 olefin affords
5α-chloro-3β,17β-dihydroxyandrost-5-en-11-one 3,17-
bis(dimethyl-t-butylsilyl) ether 3. Alkylation with
methyl lithium proceeds from the less hindered α face
10 to yield 5α-chloro-11α-methylandrostan - 3β,11β,17β-
.triol-3,17-bis(dimethyl-t-butylsilyl) ether 6. Treat-
ment of the chloro silyl ether 6 with base followed by
tetrabutyl ammonium fluoride affords 11β-methylandrost-
5-en-3β,17β-diol 7. Selective silylation yields 11β-
15 methylandrost-5-en-3β,17β-diol 3-dimethyl t-butylsilyl
ether 8. Oxidation of the C-17 alcohol in 8 yields 9 and depro-
tection of the 3-alcohol yields 11β-methylandrost-5-en-
3β-ol-17-one 10. (11β-methyl DHEA).

20 Alkylation at Carbon-16
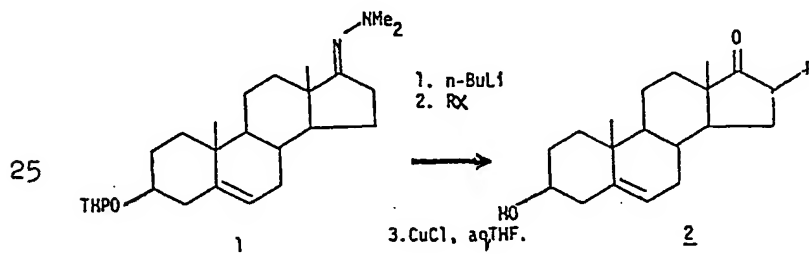
Alkylation of the 17-ketodimethylhydrazone of
DHEA 3-tetrahydropyranyl ether using n-butyl lithium as
the base followed by an alkyl halide RX, afforded the
16α-alkylated steroid. Hydrazone cleavage with cuprous

5   chloride in aqueous tetrahydrofuran led to regeneration
of the C-17 ketone and concomitant cleavage of the
tetrahydropyranyl ether resulting in the 16α-alkyl-3ß-
hydroxy-androst-5-en-17-one 2. Similarly, 3-ß, 16α-dimethyl-
androst-5-en-17-one may be prepared by alkylation of 3-ß,

10   methyl androst-5-en-17-one using this procedure to introduce
the 16α-methyl group, as illustrated by the following
procedure:

        Diisopropyl amine (1.165g, 11.5mmol) was dissolved
in dry tetrahydrofuran (30ml) at-78°C under N_3. n-Butyl

15   lithium (4.44ml of a 2.6M solution in hexane, 11.5 mmol) was
added via syringe and the solution warmed to -23°C (CO_2,
CCl_4) for .25h. 3β-methyl androst-5-ene-17-one   (3.0g,
10.4 mmol) in dry tetrahydrofuran (30ml) was added via
syringe and the solution stirred for .25h. Methyl iodide

20   (7.0g, 49.33 mmol) in dry tetrahydrofuran (30ml) was added
dropwise and the mixture stirred at room temperature for
1.5 h. The solution was quenched with saturated ammonium
chloride and the organic layer separated, dried over mag-
nesium sulfate, filtered and evaporated. The residual solid

25   was chromatographed on flash silica gel (120g) and eluted
with 1/99 (v:v) ether hexane to give 3β,16α-dimethyl
androst-5-ene-17-one   (2.3g, 74%) M.P. 109-110°C (recrys-
tallized from methanol). NMR($CDCl_3$)d 5.29(brd, J=5Hz, 1H,
H-6), 2.52(m, 1-H, H-16) 1.07 (d J=8Hz, 3H, C-16Me), .99

30   (S, 3H, C-19Me), .91(S, 3H, C-18Me). IR(kBr) 2900, 1730,
1450, 1430, 1370. Anal. calc. for $C_{21}H_{32}O$, C-83.93, H-10.73.
Found C-83.79, H-10.52. MS 300M+(100) 285(62), 282(2),
272(12), 267(17), 229(20), 217(30), 159(17).

The following procedures are illustrative of
alkenylation and alkynylation at Carbon-1.

Alkenylation (-CH = CHR) may be effected using
the vinyl analogue of the organocuprate reagent i.e.
$(CHR=CH)_2$ CuLi as in Scheme 1 above. Alkynylation
(-C≡C-R) using dialkynyl lithium cuprate is possible but
this reagent is extremely sluggish. However, using a
tri-n-butylstannyl ethylene which may be oxidized by
lead tetraacetate to an acetylene (E. J. Corey and
R. H. Wollenberg, J. Amer. Chem. Soc., 1974, 96, 5581)
affords a convenient method for the introduction of
an acetylide group. Thus using 2-tri-n-butylstannyl
ethenyl 1'-pentynyl lithium cuprate ($[C_3H_7C≡C-Cu-CH=CHSn$ $nBu_3]Li$), tri-n-butylstannylethylene is added to
the steroid. Oxidation using lead tetraacetate proceeds
with the loss of tin and affords the corresponding
acetylide. Also aluminum acetylides undergo conjugate
addition to enones.

1 Alkenylation and Alkynylation at Carbon-2
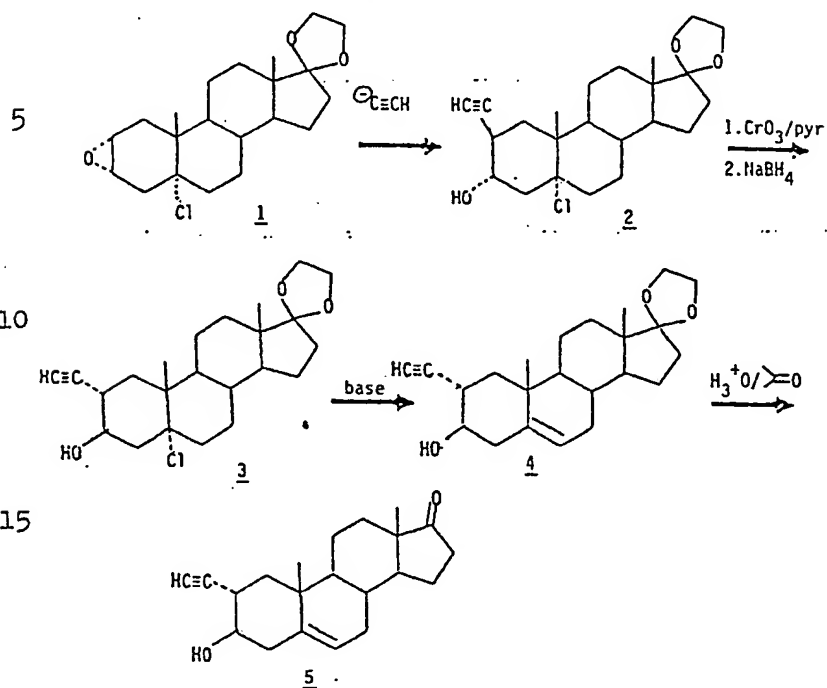
1   Reaction of 5α-chloro-2α,3α-epoxyandrostan-17-
one 17 ketal 1 with lithium acetylide ethylene diamine
complex yields 5α-chloro-2β-ethynylandrostan-3α-ol-17-
one 17-ketal 2. Epimerisation of the C-3 alcohol by
5 oxidation to the C-3 ketone (chromium trioxide/pyridine)
and reduction with sodium borohydride affords 5α-chloro-
2β-ethynylandrostan-3β-ol-17-one 17-ketal 3. Deprotec-
tion of the C-5 olefin and 17-ketone by treatment first
with base (K$_2$CO$_3$ in methanol) followed by aqueous acid
10 yields 2α-ethynyl-3β-hydroxyandrost-5-en-17-one 5. The
2α-ethenyl steroid can be synthesized from the ethynyl
derivative by careful catalytic reduction with Lindlar
catalyst to yield 2α-ethenyl-3β-hydroxyandrost-5-en-17-
one.

15 Alkenylaton and Alkynylation at Carbon-3

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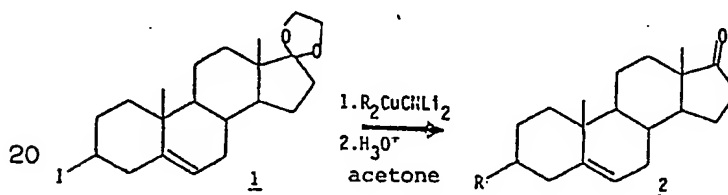
          Reaction of 3β-iodoandrost-5-en-17-one 17-ketal
25 1 with the organo cuprate reagent (R^{1}CH=CH)$_2$ Cu(C≡N)Li$_2$
generated from the appropriate vinyl lithium reagent and
cuprous cyanide, should yield the 3β-alkenylandrost-5-en-
17-one 2 (R=CH=CHR^1) following removal of the C-17-ketal.
Similarly, reaction of 1 with the tri-n-butyl stannyl
30 derivative R^1=nBu$_3$Sn yields upon oxidation with lead
tetraacetate and hydrolysis of the C-17 ketal, 3β-ethynyl-
androst-5-en-17-one 2 (R= C≡CH).

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1 Alkenylation and Alkynylation at Carbon-4

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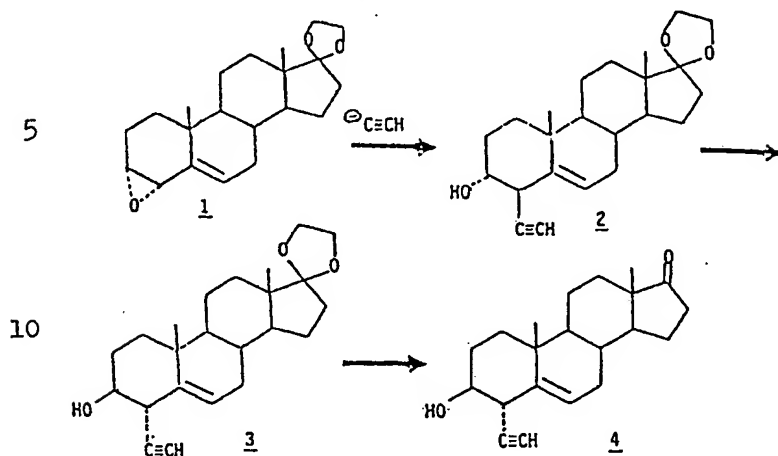
15   Reaction of 3α,4α-epoxyandrost-5-en-17-one-17-ketal
1 with lithium acetylide diethylamine complex affords
4β-ethynyl-3 α-hydroxyandrost-5-en-17-one 17-ketal 2.
Epimerisation of the C-3 alcohol by oxidation to the
C-3 ketone with chromium trioxide/pyridine followed by
20 reduction with sodium borohydride affords 4β-ethynyl-3,
β-hydroxyandrost-5-en-17-one 17-ketal 3. Careful hydroly-
sis of the C-17 ketal affords 4α-ethynyl 3β-hydroxyandrost-
5-en-17-one, 4.

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1 <u>Alkynylation at Carbon-6</u>

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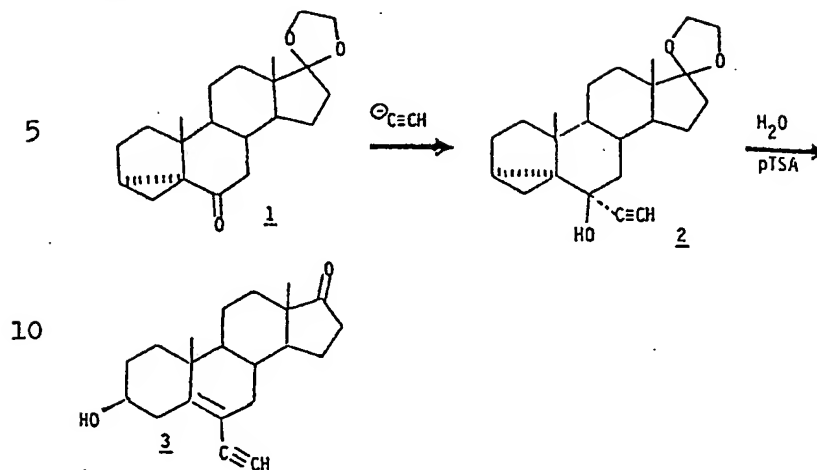
15   Treatment of 3α,5-cyclo-5-androstan-6,17-
dione 17-ketal <u>1</u>, with lithium acetylide diethyl
amine complex yields 6α-ethynyl -6β-hydroxy-3α,5-
cyclo-5α-androstan-17-one 17-ketal <u>2</u>. Reaction of <u>2</u>
with aqueous acid yields 6-ethynyl-3β-hydroxyandrost
20 5-en-17-one, <u>3</u>.

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1 Alkenylation and Alkynylation at Carbon-7
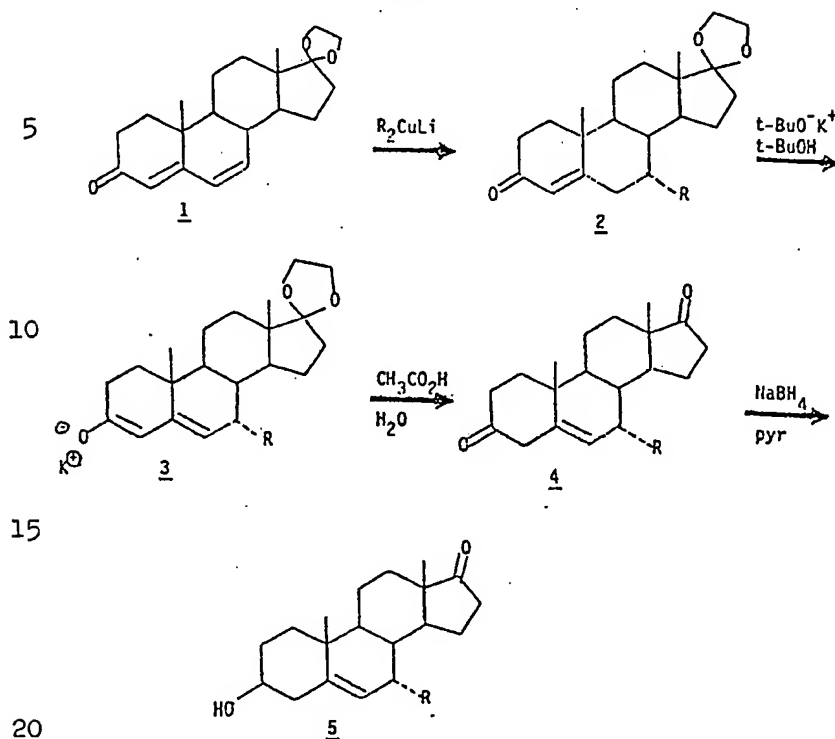
Alkenylation and alkynylation of androsta-4,6-
dien-3,17-dione 17-ketal $\underline{1}$ with $(CHR=CH)_2$ CuLi yields
the 7α-alkenyl steroid $\underline{2}$. Treatment of $\underline{2}$ with potassium
t-butoxide in t-butanol yields the dienolate $\underline{3}$ which
upon protonation with acetic acid yields 7α-alkenylandrost-
5-en-3,17-dione $\underline{4}$. Selective reduction of the C-3 ketone
using sodium borohydride in pyridine yields 3β-hydroxy-7α-
alkenyl-androst-5-en-17-one, $\underline{5}$ (R=CH=CHR[1]). Alkynylation
may be effected using 2-tri-n-butylstannyl ethenyl 1'-pen-
tynyl lithium cuprate ([$C_3H_7C≡C$-Cu-CH=CHSnBu$_3$]Li), as the
alkynylating reagent. The tri-n-butylstannylethylene
added by this reagent is oxidized using lead tetraacetate

1 resulting in the loss of tin and the formation of an
acetylide, namely 3β-hydroxy-7α-alkynylandrost-5-en-
17-one, 5 (R=C≡CH).

5 Alkenylation and Alkynylation at Carbon-11
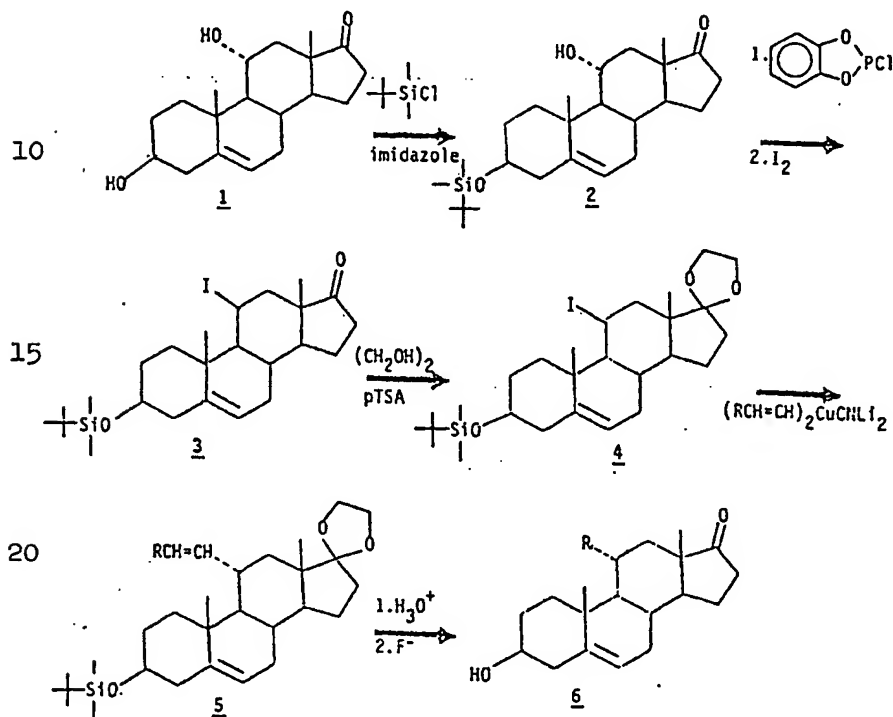
1   Reaction of the less hindered 3β-hydroxyandrost-
5-en-17-one 1 with t-butyldimethylsilyl chloride yields
the 3β-t-butyldimethylsilyl ether 2. Treatment of this
first with catechol phosphochloridate followed by dis-
5 placement with iodine yields 3β-hydroxy-11β-iodoandrost-
5-en-17-one dimethyl-t-butylsilyl ether 3. Protection
of the C-17 ketone as the 1,3-dioxolane 4 followed by
alkenylation using dialkenyl dilithio cyano cuprate,
$(RCH=CH)_2$ $CuCNLi_2$ yields 11α-alkenyl-3β-hydroxyandrost-
10 5-en-17-one t-butyldimethylsilyl ether 5. Deprotection
of the C-17 ketone and 3β alcohol affords 11α-alkenyl
3β-hydroxylandrost-5-en-17-one 6. If 6 has R=2'-tri-n-
butylstannyl ethenyl then lead tetraacetate oxidation
affords 11α-alkynyl 3β-hydroxyandrost-5-en-17-one, 6,
15 (R=C≡CH).

Alkynylation and Alkenylation at Carbon-16
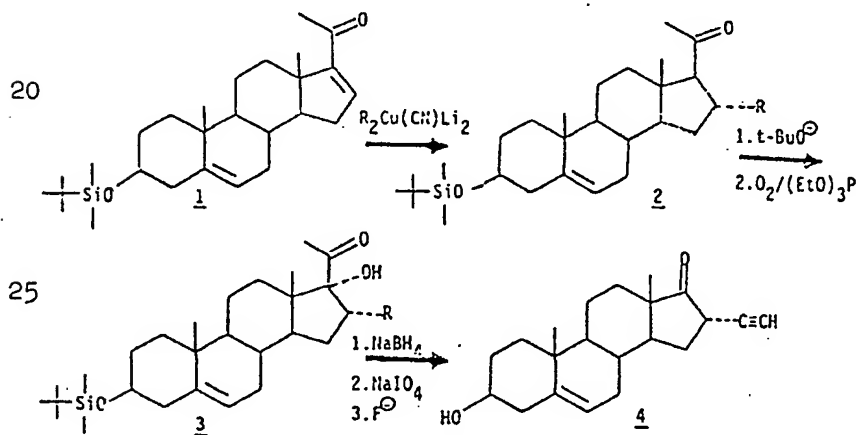
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1   Michael addition of a suitably substituted organo copper reagent such as 2-tri-n-butylstannyl ethenyl 1'-pentynyl lithium cuprate ($[C_3H_7C\equiv C$-Cu-CH=CH Sn nBu_3]Li) to 3β-hydroxypregna-5,16-dien-20-one 3-t-

5 butyl dimethylsilyl ether <u>1</u> yields a 16α-tri-n-butylstannyl ethylene (<u>2</u>, R=CH=CHSn nBu_3). Lead tetraacetate oxidation proceeds with the loss of tin and yields the correspond- ing acetylide. Treatment of <u>2</u> with t-butoxide followed by oxygen to generate a C-16α-hydroperoxide which is

10 reduced by triethylphosphite to 16α-ethynyl-3β,17α-dihy- droxy-pregna-5-en-20-one 3-t-butyldimethylsilyl ether <u>3</u>. Reduction of the C-20 ketone to an alcohol followed by cleavage of the diol with sodium periodate and depro- tection of the 3β-hydroxyl group with fluoride, yields

15 16α-ethynyl-3β-hydroxyandrost-5-en-17-one, <u>4</u>. Careful reduction of the acetylene in <u>4</u> should afford the 16α- vinyl substituted steroids. Higher homologues of these substituents may be synthesized via similar routes.

      The following procedures illustrate hydroxy-

20 lation at Carbon-1, 2, 4, 7, 11 or 16.

35
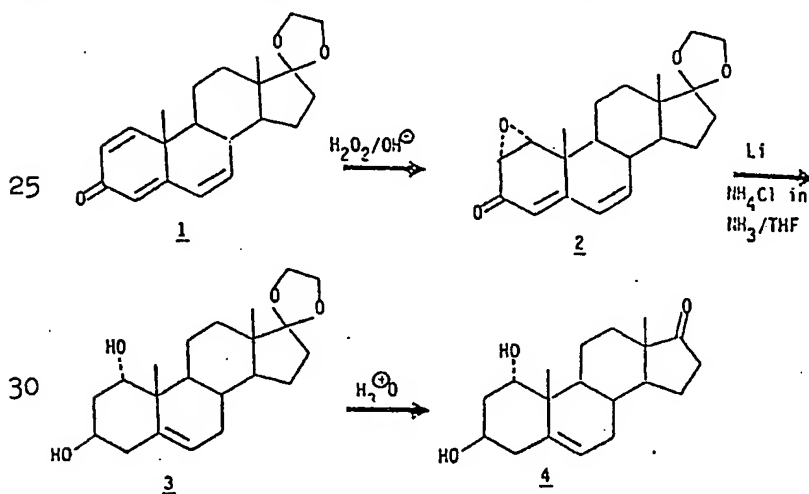
1   Alkaline hydrogen peroxide epoxidation of
androsta-1,4,6-triene-3,17-dione 17-ketal 1 with basic
hydrogen peroxide yields the 1α,2α-epoxide 2. Treat-
ment of 1α,2α-epoxyandrosta-4,6-dien-3,17-dione 17-ketal
5   2 with a large excess each of lithium metal and ammonium
chloride in ammonia-tetrahydrofuran (1:1) at reflux
leads to 1α,3β-dihydroxyandrost-5-en-17-one 17-ketal 3.
Hydrolysis of the ketal affords 1α,3β-dihydroxyandrost-
5-en-17-one, 4. Also, fermentation of DHEA with peni-
10   cillium aspergillus affords 4, i.e. penicillium asper-
gillus may be able to 1α-hydroxylate other substrates.

15
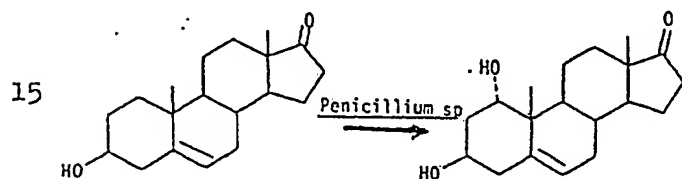
20

Dodson, R.M., Goldkamp, A.M., and Muir, R.D., JACS, 1957,
79, 3921.
Dodson, R.M., Goldkamp, A.M., and Muir, R.D., JACS, 1960,
82, 4026.
25   Penicillium hydroxylates DHEA at C-1 in the
α-position. Therefore, other substrates that look like
DHEA should by hydroxylated at C-1 by this enzyme.

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1 C-2 Hydroxylation

2α,3β-dihydroxyandrost-5-en-17-one

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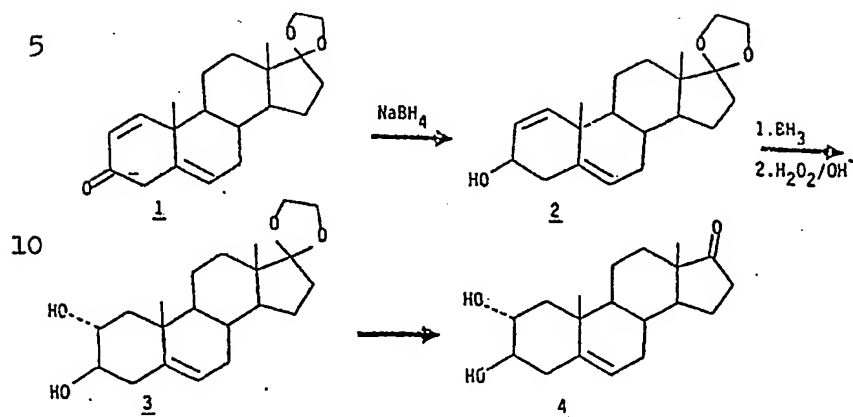
Reduction of androsta-1,5-dien-3,17-dione-17-ketal 1 with sodium borohydride yields 3β-hydroxyandrosta-1,5-diene-17-one 17-ketal 2. Hydroxylation of the C-1

20 double bond by hydroboration followed by oxidation with alkaline hydrogen peroxide affords 2α,3β-dihydroxy-androst-5-en-17-one 17-ketal 3. Deprotection of the C-17 ketone with aqueous acid yields 2α,3β-dihydroxy-androst-5-en-17-one, 4.

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Carbon-4 Hydroxylation
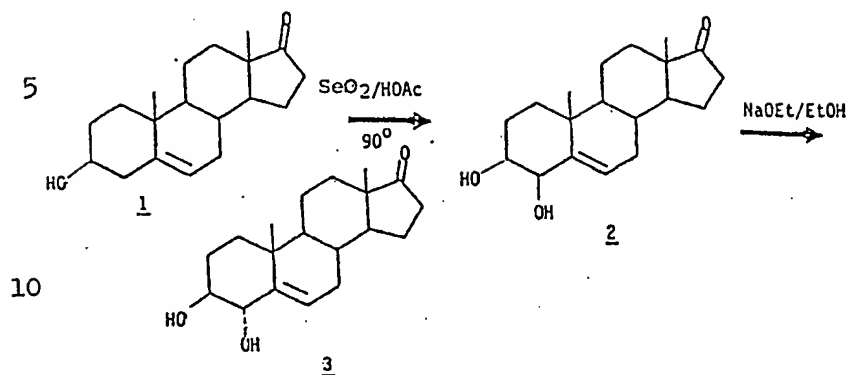
15   Selenium dioxide oxidation of 3β-hydroxyandrost-5-en-17-one yields $3\beta,4\beta$-dihydroxyandrost-5-en-17-one $\underline{2}$. The axial C-4 alcohol may be epimerized to the equatorial position by reaction with sodium ethoxide in ethanol to yield $3\beta,4\alpha$-dihydroxyandrost-5-en-17-one, $\underline{3}$.

1 <u>Carbon-7 Hydroxylation</u>

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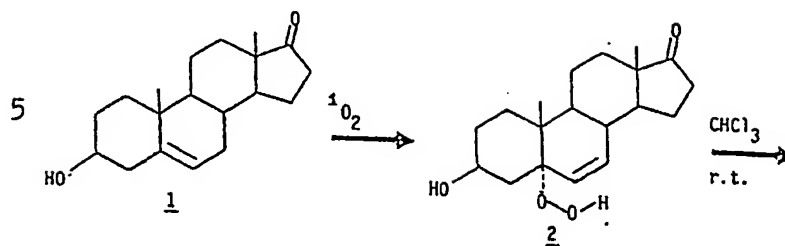
25 3β-Hydroxyandrost-5-en-17-one (DHEA) <u>1</u> reacts
with singlet oxygen to yield 5α-hydroperoxy-3β-hydroxy-
androst-6-en-17-one <u>2</u>. This hydroperoxide undergoes a
rearrangement when in chloroform solution to yield 7α-
hydroperoxy-3β-hydroxyandrost-5-en-17-one, <u>3</u>. Catalytic
30 reduction of the hydroperoxide yields 3β,7α-dihydroxy-
androst-5-en-17-one, <u>4</u>.

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1 ## Carbon-11 Hydroxylation
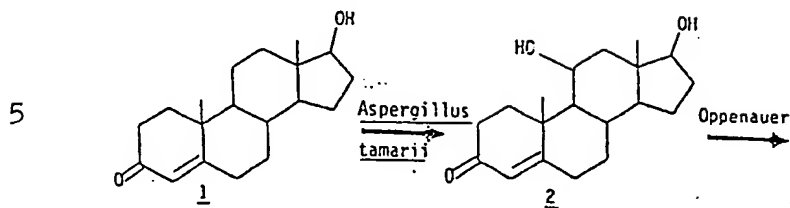
10 D.R. Brannon, J. Martin, A.C. Ochlschlager, N.N. Durham, and L.H. Zalkow, J. Org. Chem. 1965. 30, 760.
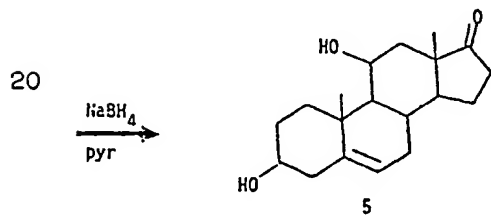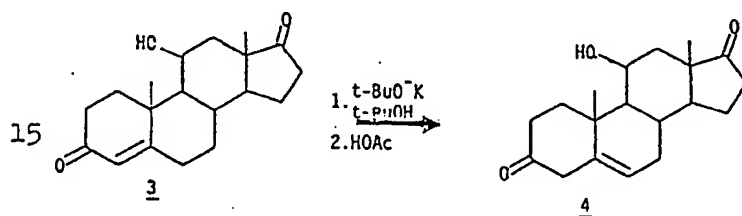
Hydroxylation of testosterone 1 at Carbon-11
25 using Aspergillus tamarii affords 11β,17β-dihydroxyan-
drost-4-en-3-one 2. Oppenauer oxidation of 2 oxidizes
the 17β-alcohol in the presence of the hindered 11β-
hydroxyl group to yield 11β-hydroxyandrost-4-en-3,17-dione,
3. Migration of the double bond out of conjunction by
30 treatment with potassium t-butoxide followed by protona-
tion with acetic acid yields 11β-hydroxyandrost-5-en-3,
17-dione 4. Selective reduction of 4 yields 3β,11β-dihy-
droxyandrost-5-en-17-one, 5.

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1   <u>Hydroxylation at Carbon-16</u>

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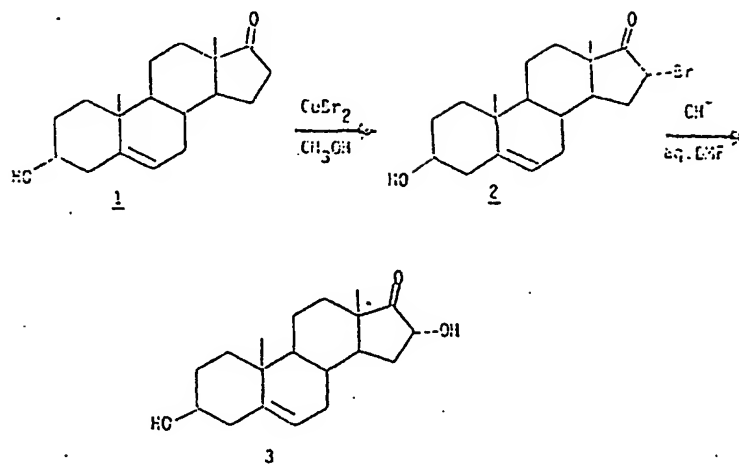
15   Bromination of DHEA (<u>1</u>) with cupric bromide
     yields 16∝-bromo-DHEA, <u>2</u>.   Treatment of the bromo
     ketone <u>2</u> with sodium hydroxide in aqueous dimethyl-
     formamide gave 3β ,16∝-dihydroxyandros -5-en-17-one, <u>3</u>.
     See M. Numazawa, M. Nagaoka, Y. Osawa, J. Org. Chem. 1982,
20   47, 4024. Similarly 3-β-methyl-16∝ hydroxy androst-5-
     en-17-one may be prepared by hydroxylation of 3-β methyl
     androst-5-en-17-one using this procedure to introduce
     the 16∝-hydroxy group, as illustrated by the following
     procedure:
25   To prepare 3 β-methyl-16∝-bromo-androst-5-
     ene-17-one (Ref: E. R. Glazier, J. Org. Chem. (1962)
     <u>27</u> 2937; M. Numazawa & Y. Osawa, J. Org. Chem. Steriods
     (1978) <u>32</u>, 519; M. Numazawa, M. Nagaoka & Y. Osawa, J.
     Org. Chem. (1982), <u>47</u> 4024).
30   3 β-methyl androst-5-ene-17-one (4.0g, 14 mmol)
     and $CuBr_2$ (9.4g, 42mmol) were dissolved in methanol (250ml)
     and refluxed for 24h. The hot solution was filtered to
     remove the white precipitate and the filtrate cooled to

35

1   yield 3.1g (61%) 3β methyl-16α-bromo androst-5-ene-17-one.
An analytical sample was prepared by passing an ether
solution of the steroid through a small plug of neutral
alumina. Evaporation and recrystallization from methanol
5   gave white needles, M.P. 193-195°C. Anal. calc. for
$C_{20}H_{29}OBr$, C-65.7, H-8.0. Found C-65.54, H-8.11 NMR
$(CDCl_3)$ d 5.30 (brd, 1H, H-6), 4.52 (t, 1H, 16β-H) .98
(S, 3H, C-19Mc), .90(S, 3H, C-18Me) IR(KBr) 2910, 1735,
1445, 1365, 1020.

10   3β-methyl-16α-bromo androst-5-ene-17-one
(1g, 2.74 mmol) was dissolved in dimethylformamide (90ml).
Sodium hydroxide (165 mg, 4.1 mmol) in water (10ml) was
added and the solution stirred for 2 h. at room tempera-
ture. The solution was poured into 1% HCl (200 ml) and
15   extracted with ethyl acetate (2 X 50 ml). The organic
layer was washed with 5% sodium bicarbonate, water, then
dried over magnesium sulfate and filtered. Evaporation
gave a pale yellow solid which was chromatographed on
flash silica gel and eluted with ether/hexane (10/90).
20   Recrystallization from ether gave 3β-methyl-16α-hydroxy
androst-5-ene-17-one (0.4g, 50%). M.P. 166-168°C. Anal.
calc. for $C_{20}H_{30}O_2$, C-79.42, H-9.99. Found C-79.24,
H-10.04 NMR (d-6-DMSO) d 5.30(brd, 1H, H-6), 4.38(t, 1H,
16β-H) [IR(KBr) 3440, 2900, 1735, 1445, 1365, 1010.]
25   2.0-1.85(complex).
        The following procedures are representative of
procedures for halogenation at Carbon-1, 2, 3, 4, 6, 7,
11 or 16.

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X = Cl,Br,I
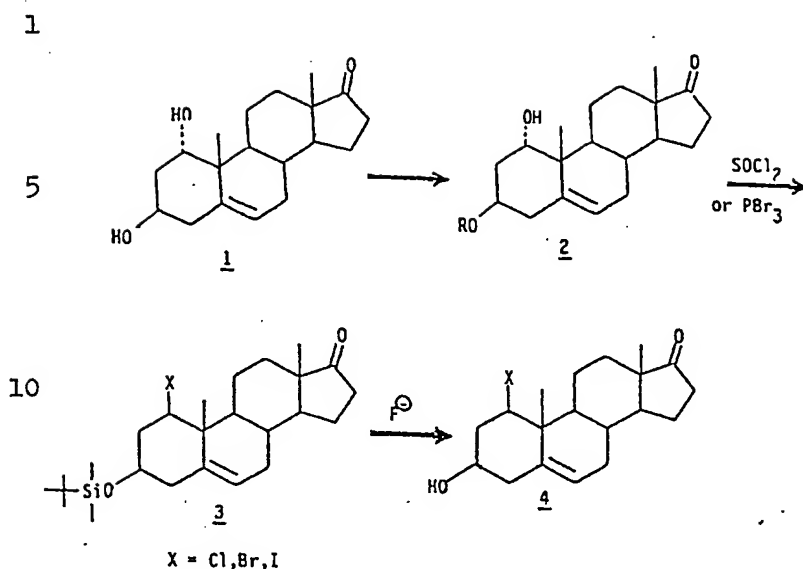
20 Selective protection of the Carbon-3 hydroxyl in the presence of the 1α-hydroxyl group should yield $\underline{2}$. For example, 1α,3β-dihydroxyandrost-5-en-17-one $\underline{1}$ reacts with t-butyl-dimethyl silyl chloride in the presence of imidazole using dimethylformamide as a solvent to yield 1α,3β-dihydroxyandrost-5-en-17-one 3t-butyldimethylsilyl

25 ether, $\underline{2}$. Reaction of $\underline{2}$ with thionyl chloride, or phosphorous tribromide or catechol phosphochloridate followed by iodine yields the corresponding 1β-chloro, bromo or iodo derivatives $\underline{3}$. Reaction of $\underline{3}$ (R=Cl, Br, I) with tetrabutyl ammonium fluoride yields 1β,-halo-3β-hydroxy

30 androst 5-en-17-one, $\underline{4}$ (R=Cl, Br or I). The fluoride ($\underline{4}$, R=F) may be synthesized via a similar route using

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1 an ester as the protecting group at C-3 and reacting the
1α-hydroxyl group with diethyl (2-chloro-1,1,2-trifluoro-
ethyl)amine. Hydrolysis should yield 1,β-fluoro-3β-
hydroxyandrost-5-en-17-one, 4, R=F.

5 Halogenation at Carbon-2
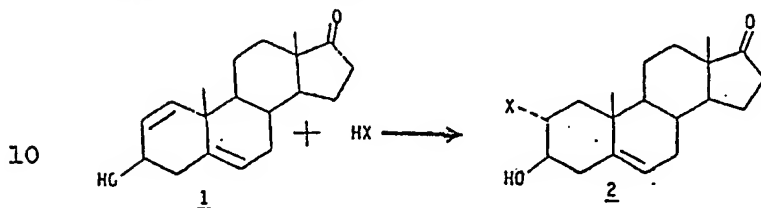
.Addition of HX across the C-1 double bond in
3β-hydroxyandrosta-1,5-diene-17-one, 1, yields a mixture
15 of the C-1 and C-2 halogenated steroids. Separation
affords 2-halo-3β-hydroxyandrost-5-en-17-one (2, R=F,
Cl, Br, I).

Halogenation at Carbon-3

20
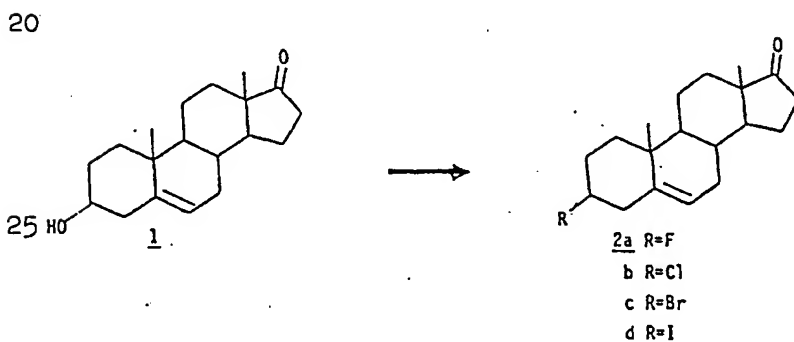
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1   Reaction of 3β-hydroxyandrost-5-en-17-one <u>1</u>
with diethyl (2-chloro-1,1,2-trifluoroethyl) amine
yields 3β-fluoroandrost-5-en-17-one <u>1</u>. Reaction of <u>1</u>
with thionyl chloride yields 3β-chloroandrost-5-en-17-

5   one, <u>2b</u>. Reaction of <u>1</u> with phosphorus tribromide
yields 3β-bromoandrost-5-en-17-one, <u>2c</u>. Reaction of
<u>1</u> with catechol phosphochloridate followed by iodine
yields 3β-iodoandrost-5-en-17-one <u>2d</u>.

Halogenation at Carbon-4
10

	<u>2a</u> X=F
	<u>b</u> X=Cl
	<u>c</u> X=Br
	<u>d</u> X=I
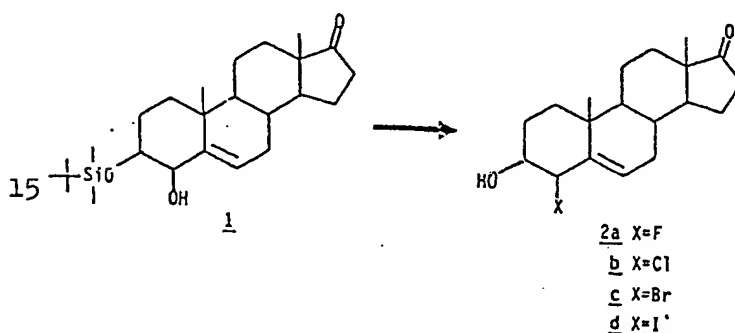
20
          With the 3β-hydroxyl group protected as its
t-butyl-dimethylsilyl ether the C-4 hydroxyl may be
chlorinated using thionyl chloride. Treatment with
fluoride ion cleaves the silyl ether to yield 4-chloro-

25 3β-hydroxyandrost-5-en-17-one, <u>2b</u>. Reaction of 3,4-
dihydroxyandrost-5-en-17-one 3-t-butyldimethylsilyl
ether <u>1</u> with catechol phosphochloridate, followed by
displacement with bromide ion and cleavage of the silyl
ether with fluoride ion yields 4-bromo-3β-hydroxyandrost-

30 5-en-17-one, <u>2c</u>. Reaction of <u>1</u> with catechol phospho-
chloridate, followed by iodine and cleavage of the silyl
ether with fluoride yields 4-iodo-3β-hydroxyandrost-5-en-
17-one, <u>2d</u>. Fluorination of 3,4-dihydroxyandrost-5-en-
one 3-acetate with diethyl (2-chloro-1,1,2-trifluoroethyl)

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1 amine followed by hydrolysis of the ester yields 4-fluoro-
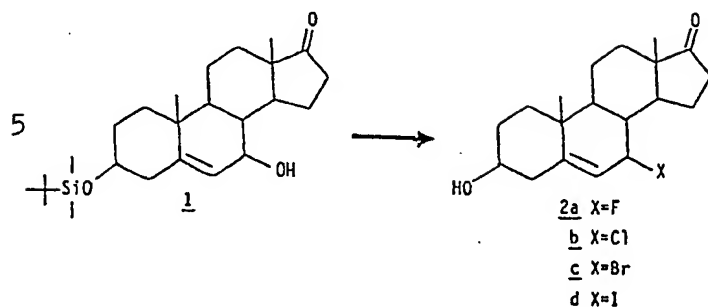3β-hydroxyandrost-5-en-17-one, 2a.

Halogenation at Carbon-6

5

10 1

2a X=F c X=Br
b X=Cl d X=I

15 3

1.NaBH₄
2.⊖OH/MeOH

4

1.Si—Cl
2.CrO₃/pyr

20

F⊖

5a X=F   5c X=Br
b X=Br   d X=I

6

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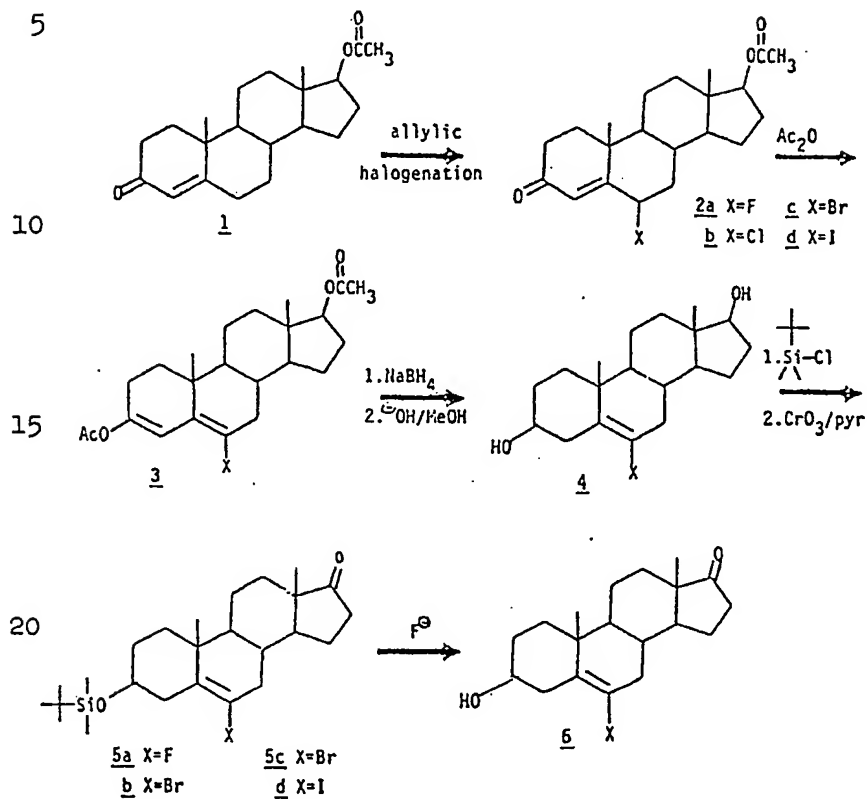
1   Allylic bromination of 17β-hydroxyandrost-4-
en-3-one 17-acetate 1 using N-bromosuccinimide together
with a radical initiator such as light or benzoyl
peroxides or aliphatic azo compounds [RR'C(CN)-N=N-C(CN)
5 RR'] e.g. azobisisobutyronitrile yields 6β-bromo-17β-
hydroxyandrost-4-en-3-one 17-acetate, 2. Allylic chlori-
nation of 1 using sulfuryl chloride together with a
radical initiator such as light or benzoyl peroxide or
aliphatic azo compounds yields 6β-chloro-17β-hydroxy-
10 androst-4-en-3-one 17-acetate, 2c. Allylic iodination
of 1 using mercuric iodide and light yields 6β-iodo-
17β-hydroxyandrost-4-en-3-one-17-acetate, 2d. Acety-
lation of 2 with acetic anhydride and p-toluene sulfonic
acid in toluene yields 6-halo-3,17β-dihydroxyandrosta-
15 3,5-diene 3,17-diacetate 3. Sodium borohydride reduction
of 3 followed by basic hydrolysis of the C-17 acetate
yields 6-haloandrost-5-en-3β,17β-diol, 4. Selective
protection of the C-3 hydroxyl group as its t-butyldi-
methylsilyl ether followed by chromium trioxide oxida-
20 tion of the C-17-hydroxyl group yields 6-halo-3β-hydroxy-
androst-5-en-17-one 3-t-butyldimethylsilyl ether 5.
Treatment of 5 with fluoride ion yields 6-halo-3β-hydroxy-
androst-5-en-17-one, 6. The C-6 fluoro analogue may be
synthesized from the C-6 bromo diacetate, 3c, by treat-
25 ment with silver fluoride. Reaction of 6-fluoro-3,17β-
dihydroxyandrosta-3,5-diene-3,17-diacetate, 3a, with
sodium borohydride and following the above sequence
yields, 6-fluoro-3β-hydroxyandrost-5-en-17-one, 6a.

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1 Halogenation at Carbon-7
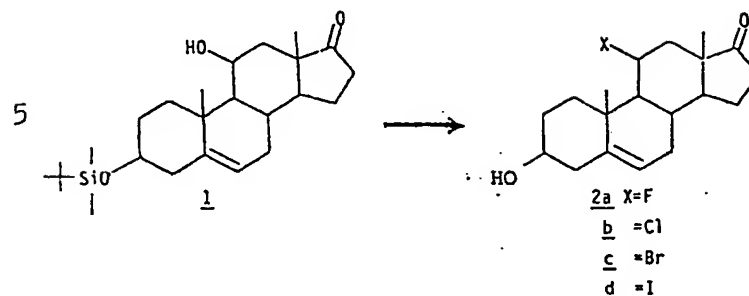

Reaction of 3β,7-dihydroxyandrost-5-en-17-one-3-t-butyldimethylsilyl ether 1 with thionyl chloride yields the C-7 chloro-steroid. Deprotection of the 3β-hydroxyl group affords 7-chloro-3β-hydroxyandrost-5-en-17-one, 2b. Reaction of 1 with catechol phosphochloridate followed by displacement with bromide ion and deprotection yields 7-bromo-3β-hydroxyandrost-5-en-17-one, 2c. Similarly reaction of 1 with catechol phosphochloridate followed by displacement with iodine and deprotection yields 7-iodo-3β-hydroxyandrost-5-en-17-one, 2d. Fluorination of 3β,7-dihydroxyandrost-5-en-17-one 3-acetate with diethyl (2-chloro-1,1,2-tri-fluoro-ethyl) amine followed by hydrolysis of the ester yields 7-fluoro-3β-hydroxyandrost-5-en-17-one, 2a.

1   Halogenation at Carbon-11

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        Reaction of 3β,11-dihydroxyandrost-5-en-17-
one 3-t-butyldimethylsilyl ether 1 with·thionyl chlo-
ride yields the C-11 chloro steroid. Deprotection of
the 3β-hydroxyl group affords 11-chloro-3β-hydroxyan-
15 drost-5-en-17-one, 2b. Reaction of 1 with catechol
phosphochloridate followed by displacement with bro-
mide ion and deprotection yields 11-bromo-3β-hydroxy-
androst-5-en-17-one, 2c. Similarly reaction of 1 with
catechol phosphochloridate followed by displacement
20 with iodine and deprotection yields 11-iodo-3β,
hydroxyandrost-5-en-17-one, 2d. Fluorination of...
3β,11-dihydroxyandrost-5-en-17-one 3-acetate with
diethyl (2-chloro-1,1,2-trifluoroethyl)amine followed
by hydrolysis of the ester yields 11-fluoro-3β-hydroxy-
25 androst-5-en-17-one, 2a.

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1 <u>Halogenation at Carbon-16</u>
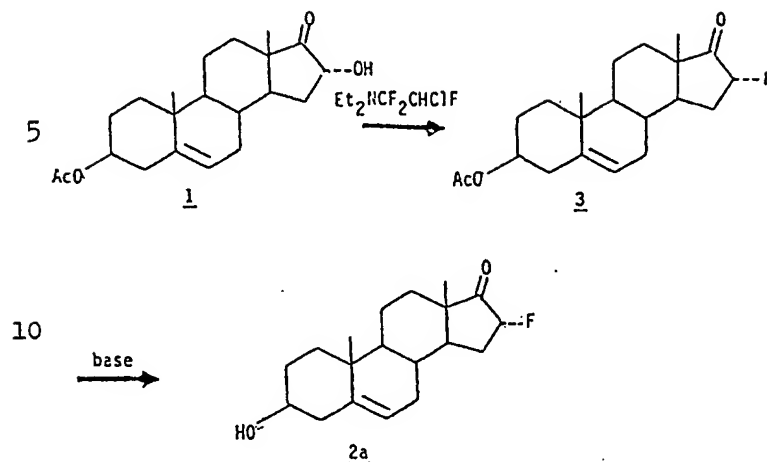
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Reaction of 3β,16α-dihydroxyandrost-5-en-17-
15 one 3β-acetate <u>1</u> with diethyl (2-chloro-1,1,2-trifluoro-
ethyl)amine affords 16α-fluoro-3β-hydroxyandrost-5-en-
17-one 3-acetate <u>3</u>. Hydrolysis of the ester with base
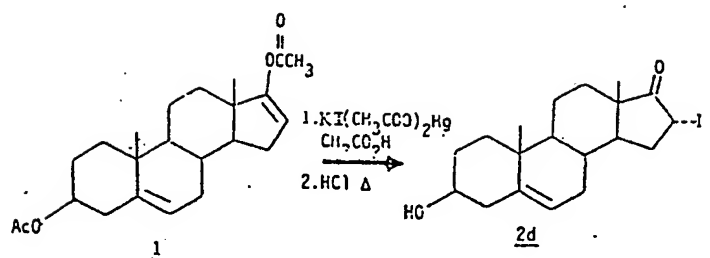yields 16α-fluoro-3β-hydroxyandrost-5-en-17-one, <u>2a</u>.

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25
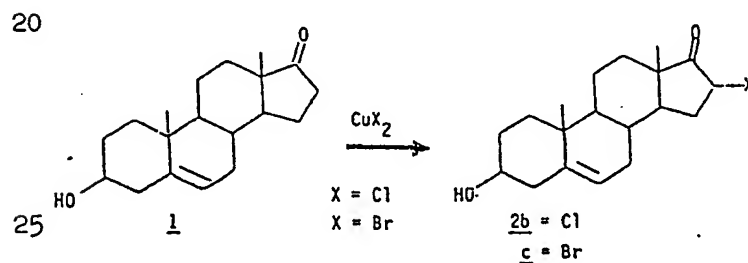
Reaction of 3β-hydroxyandrost-5-en-17-one <u>1</u>
with cupric bromide yields 16α-bromo-3β-hydroxyandrost-
5-en-17-one, <u>2c</u>[1]. Similarly reaction of <u>1</u> with cupric
30 chloride yields 16α-chloro-3β-hydroxyandrost-5-en-17-
one, <u>2b</u>.

_____

[1]E. R. Glazier J. Org. Chem. 1962, <u>27</u>, 4397

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10   Reaction of $3\beta,17$-dihydroxyandrosta-5,16-diene 17-acetate $\underline{1}$ with mercuric acetate followed by treatment with potassium iodide yielded the C-17 α iodide which hydrolyses with acid to yield 3β-hydroxy-16α-iodoandrost-5-en-17-one, $\underline{2d}$. Reaction of $\underline{2d}$ with silver fluoride

15 yields 3β-hydroxy-16α-fluoroandrost-5-en-17-one, $\underline{2a}$.

     The following procedures are illustrative for the preparation of compounds of the present invention encompassed by the structure:

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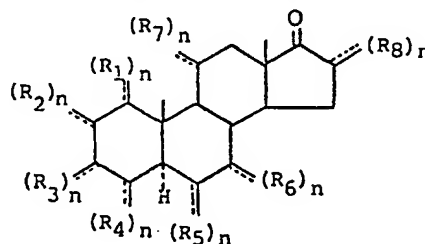
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wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8 and n are as defined hereinbefore.

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1   Catalytic hydrogenation of 3β-substituted
     androst-5-enes yields almost exclusively 3β-substituted
     5α-androstanes (for references see J. R. Lewis and C. W.
     Shoppee, J. Chem. Soc. 1955, 1365). Therefore all the
5   syntheses of the substituted androst-5-enes described
     above can be used for the synthesis of the substituted
     5α-androstanes, except those molecules which contain
     reducible double bonds such as the ethenyl and alkynyl
     derivatives. For these molecules the following synthe-
10   ses are described.

          Firstly an example of catalytic hydrogenation
     for the synthesis of 5α-androstanes from androst-5-enes
     is the synthesis of 3β-methyl-5α-androstan-17-one 2 from
     3β-methylandrost-5-en-17-one 1. 3β-Methylandrost-5-en-
15   17-one 1 (400 mg), prepared as described previously was
     dissolved in glacial acetic acid (80 ml). Palladium on
     carbon (10%, 100 mg) was added and the solution maintained
     under an atmosphere of hydrogen. When hydrogen uptake
     ceased, the solution was filtered through celite and
20   evaporated to give solid which was recrystallized from
     methanol to yield 3β-methyl-5α-androstan-17-one, 2,
     (320 mg, 80% yield). MP 107-108°C, ^{1}H NMR (CDCl$_3$)δ 0.86
     (d, 3H, J-5Hz, methyl at C-3), 0.85 (s, 3H, C-19 Me),
     0.79 (s, 3H, C-18 Me).
25   Anal Calc for C$_{20}$H$_{32}$O: C, 83.26%, H 11.18%
               Found: C, 82.99%, H 11.35%

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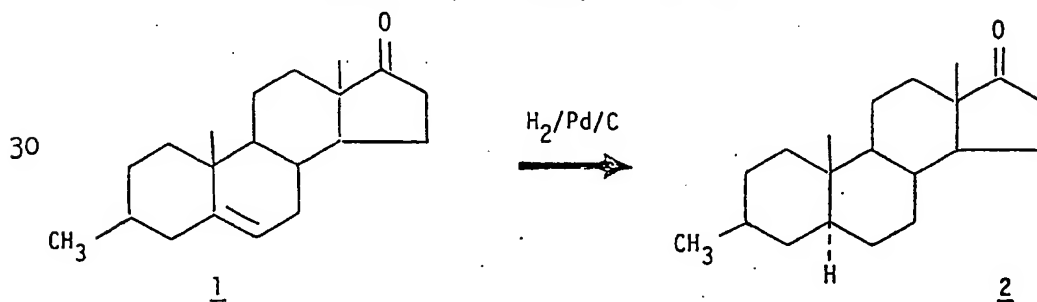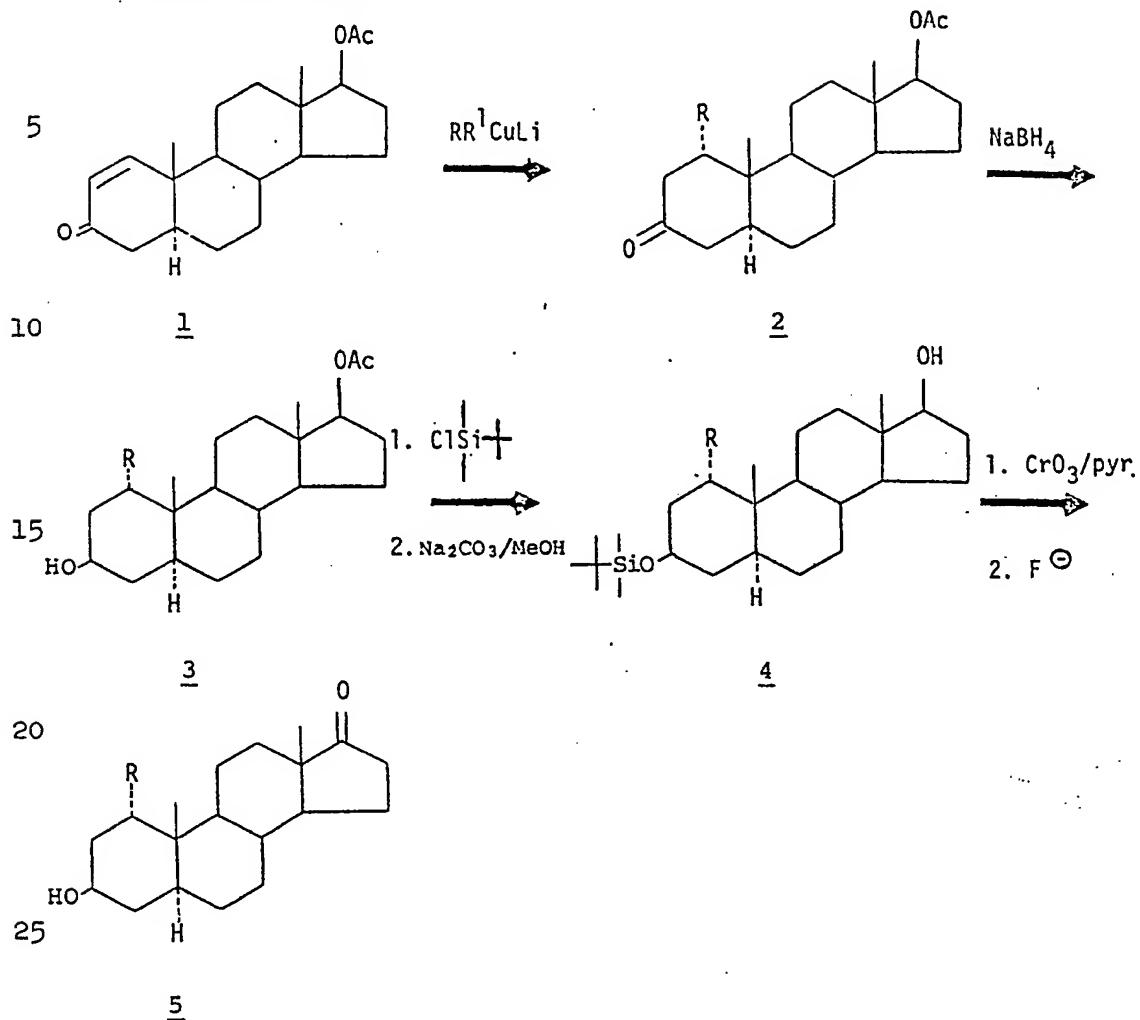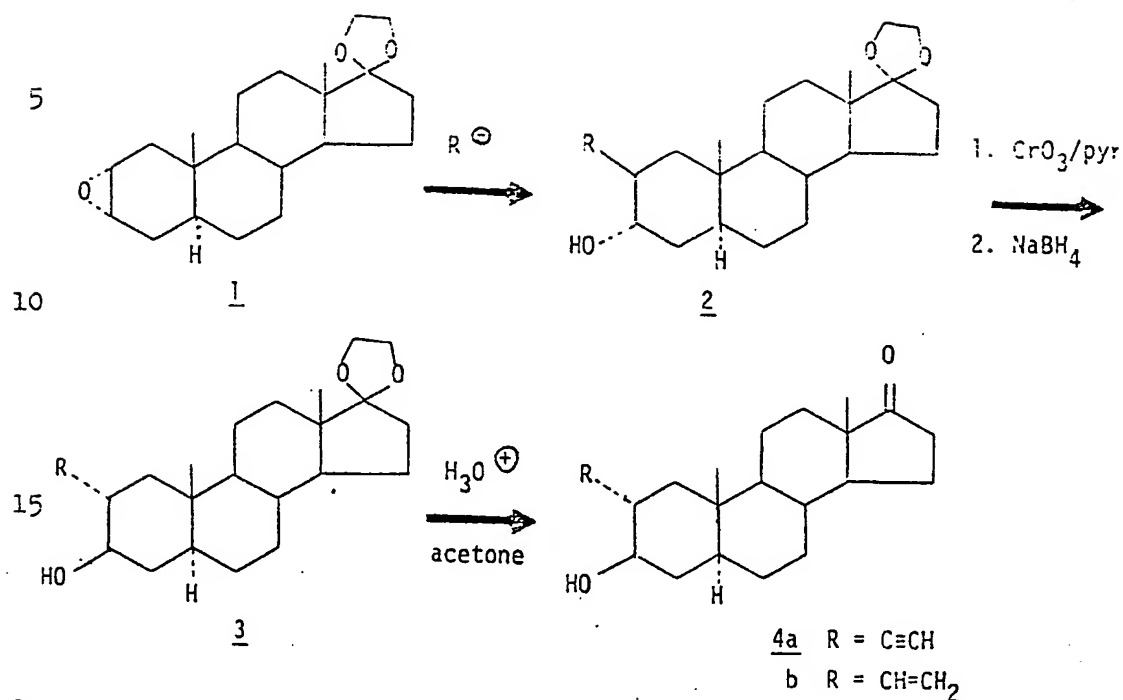
1   The following procedures are illustrative
for alkenylation and alkynylation at carbon-1.
       Michael addition to 17β-hydroxy-5α-androst-1-
en-3-one 17-acetate, 1, using a dialkenyl lithium cuprate,
5 (RR^{1}CuLi, R = R^1= CH=CHR^2) yields the 1α-alkenyl-17β-hydroxy-
5α-androstan-3-one 17-acetate 2. Reduction of the C-3
ketone in 2 yields the 3β-hydroxy steroid 3. Protection
of the 3β-hydroxyl group as a dimethyl-t-butylsilyl ether
followed by hydrolysis of the C-17 acetate yields 1α-
10 alkenyl-3β, 17β-dihydroxy-5α-androstan 3-dimethyl-t-
butylsilyl ether, 4. Oxidation of the C-17-hydroxyl group
and deprotection of the 3β-hydroxyl group with fluoride
ion affords 1α-alkenyl-3β-hydroxy-5α-androstan-17-one, 5.
(R= CH=CHR' where R' = alkyl). Alkynylation, to prepare
15 5 (R= C≡CR' where R' = alkyl) maybe carried out using
the above procedure but with a different organo cuprate
reagent. Using 2-tri-n-butylstannyl ethenyl 1'-pentynyl
lithium cuprate (R R'CuLi is equivalent to [C$_3$H$_7$C≡C-Cu-
CH=CHSnnBu$_3$]Li), (E. J. Corey and R. H. Wollenberg,
20 J. Amer. Chem. Soc. 1974, 96, 5581), tri-n-butylstannyl-
ethylene is added to 1 to yield 2 with R = CH=CHSnn-Bu$_3$.
Oxidation using lead tetraacetate proceeds with loss of
tin and affords the corresponding acetylide 2, (R= C≡CH).
Following through the reaction sequence as above yields
25 1α-ethynyl-3β-hydroxy-5α-androstan-17-one 5, R = C≡CH.

30

35

1 Alkenylation and Alkynylation at Carbon-1.

5

1

RR¹CuLi →

RR^1CuLi →

2

NaBH₄ →

10

15

3

1. ClSi—⟨⟩
2. Na₂CO₃/MeOH →

4

1. CrO₃/pyr.
2. F⊖

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Alkenylation and Alkynylation at Carbon-2.

5

10   1   2

15


20   3   4a   R = C≡CH
          b   R = CH=CH$_2$

Reaction of 2α, 3α-epoxy-5α-androstan-17-one
17-ketal $\underline{1}$ with lithium acetylide ethylene diamine
complex yields 2β-ethynyl-5α-androstan-3 -ol-17-one 17-
25 ketal $\underline{2}$, (R = C≡CH). Epimerization of the C-3 alcohol
by oxidation to the C-3 ketone (chromium trioxide/pyri-
dine) and reduction with sodium borohydride affords
2β-ethynyl-5α-androstan-3β-ol-17-one 17-ketal $\underline{3}$ (R = C≡CH).
Deprotection of the 17-ketone by treatment with aqueous
30 acid yields 2α-ethynyl-5α-androstan-3β-ol-17-one $\underline{4a}$.
The 2α-ethenyl steroid can be synthesized from the
ethynyl derivative by careful catalytic reduction with
Lindlar catalyst to yield 2α-ethenyl-3β-hydroxy-5α-andro-
stan-17-one, $\underline{4b}$.

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1　<u>Alkenylation and Alkynylation at Carbon-3.</u>
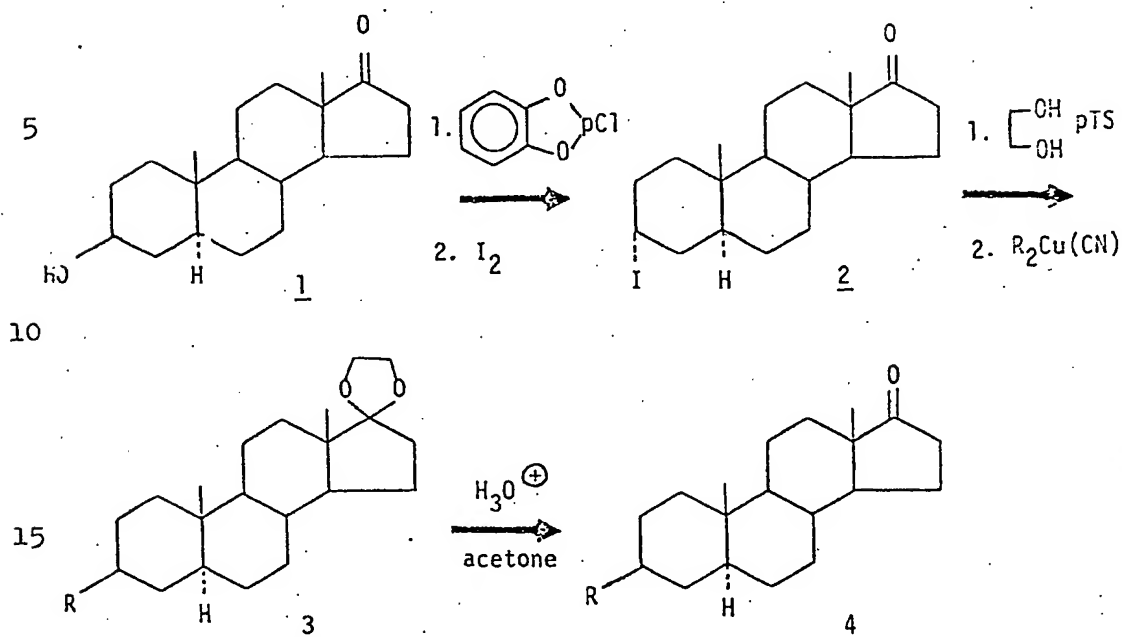
Reaction of 3β-hydroxy-5α-androstan-17-one <u>1</u>
with catechol phosphochloridate followed by iodine
affords 3α-iodo-5α-androstan-17-one, <u>2</u>.　Protection of
the C-17 ketone as a ketal followed by nucleophilic
displacement of the C-3α-iodo group yields the 3β-sub-
stituted steroid <u>3</u>.　For alkenylation at C-3, such as
introduction of a vinyl group, i.e. <u>3</u> (R = $CH=CH_2$),
divinyl cyano dilithio cuprate, $[(CH_2=CH)_2 Cu(CN)]Li_2$
is used.　Hydrolysis of the C-17 ketal yields 3β-
ethenyl-5α-androstan-17-one, <u>4</u> (R = $CH=CH_2$).　For alky-
nylation 2-tri-n-butylstannyl ethenyl 1'-pentynyl cyano
dilithio cuprate, $[C_3H_7C≡C-Cu-CH=CHSn-nBu_3(CN)]Li_2$,
yields the 3β-ethynyl derivative <u>3</u> (R = C≡CH).　Hydroly-
sis of the C-17 ketal yields 3β-ethynyl-5α-androstan-17-
one, <u>4</u>, (R = C≡CH).

1 Alkenylation and Alkynylation at Carbon-4.

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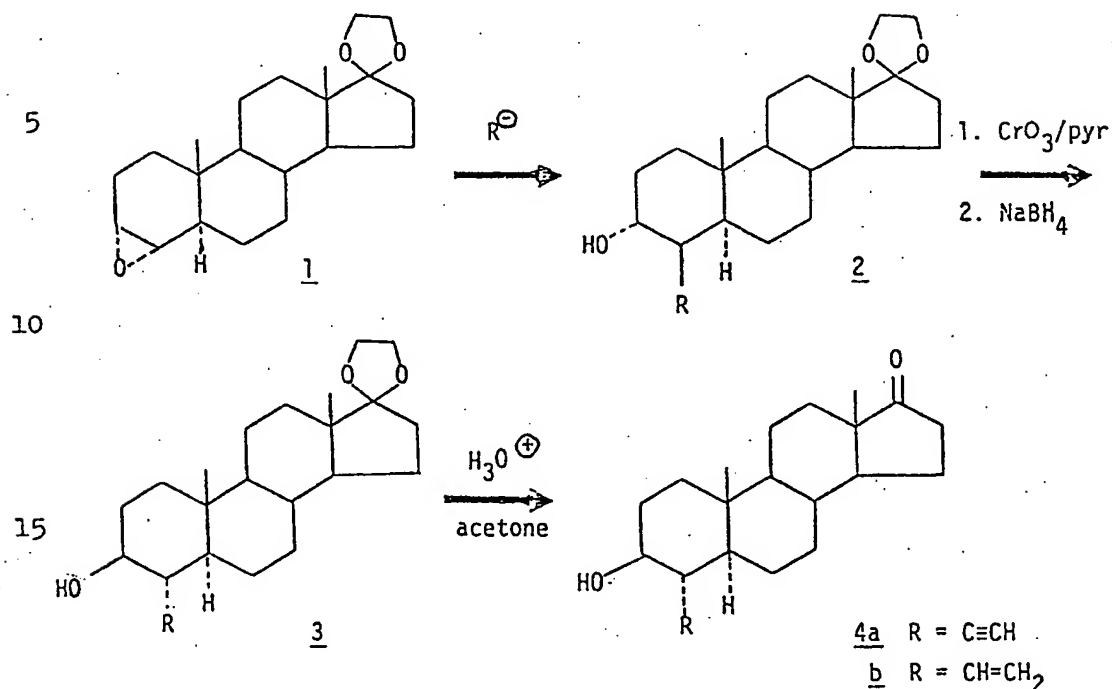
    Reaction of 3α,4α-epoxy-5α-androstan-17-one
17-ketal **1** with lithium acetylide diethylamine complex
affords 4β-ethynyl-3α-hydroxy-5α-androstan-17-one 17-
ketal **2** (R = C≡CH). Epimerization of the C-3 alcohol
by oxidation to the C-3 ketone with chromium trioxide/
25
pyridine followed by reduction with sodium borohydride
affords 4α-ethynyl-3β-hydroxy-5α-androstan-17-one 17-
ketal **3** (R = C≡CH). Careful hydrolysis of the C-17
ketal affords 4α-ethynyl-3β-hydroxy-5α-androstan-17-
one, **4a** (R = C≡CH). The 4α-ethenyl derivative, **4b**, can
30
be synthesized from the ethynyl derivative, **4a**, by
careful catalytic reduction with Lindlar catalyst or
metal ammonia reduction to yield 4α-ethenyl-3β-hydroxy-
5α-androstan-17-one, **4b**.

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1 Alkenylation and Alkynylation at Carbon-6.

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$R^\ominus$

$H_3O^\ominus$
acetone

10
1

2

15

1. Li/NH$_3$

2. NaBH$_4$

1. Ac$_2$O/pyr

2. F$^\ominus$

3

4

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1. CrO$_3$/pyr

2. Na$_2$CO$_3$/MeOH

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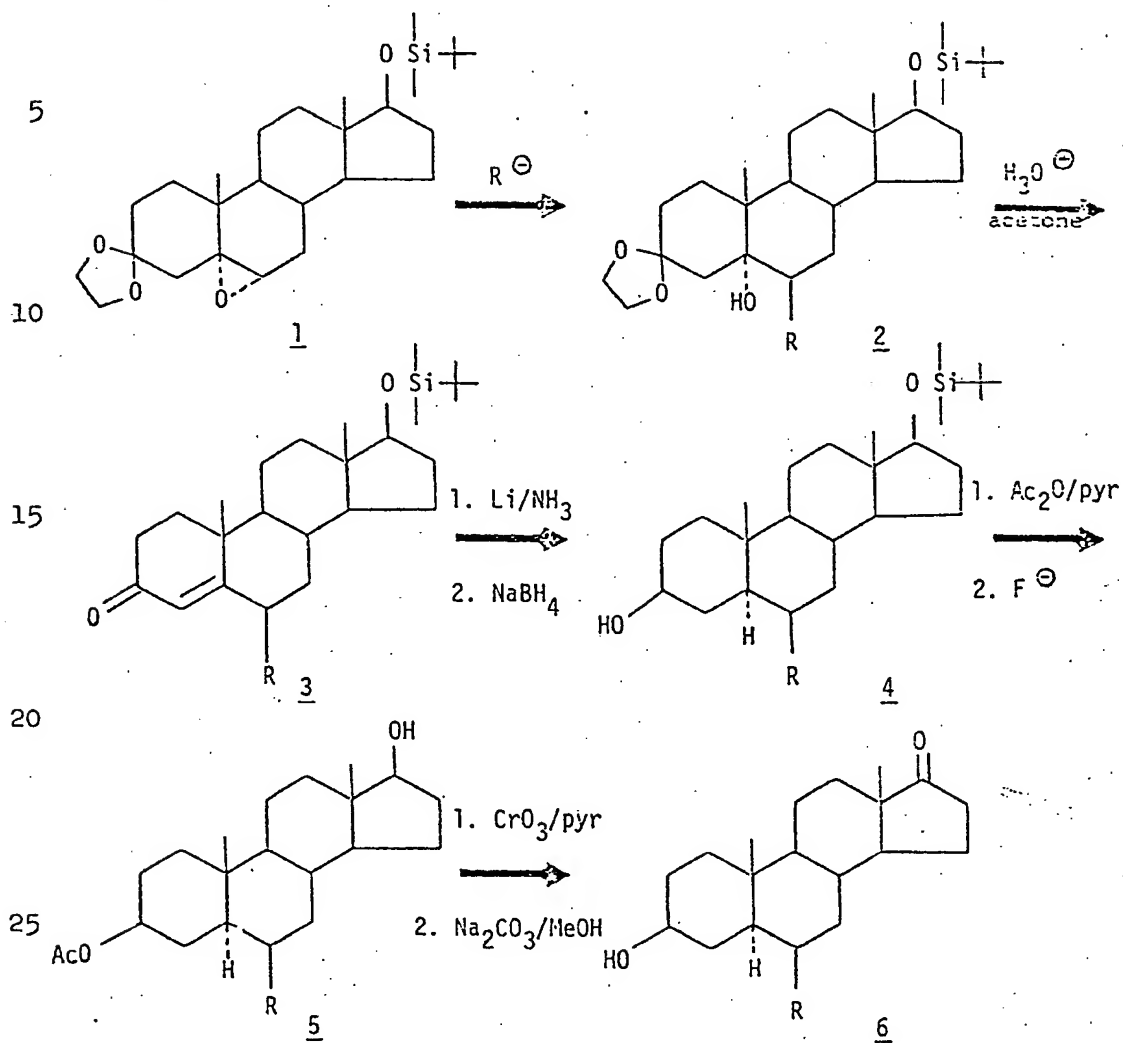
Reaction of 5α,6α-epoxy-3β-dimethyl-t-butyl-
silyl-oxyandrostan-3-one 3-ketal 1, with lithium acety-
lide-ethylenediamine complex yields 6β-ethynyl-5α-hydroxy-
5 3β-dimethyl-t-butylsilyloxyandrostan-3-one 3-ketal, 2.
Hydrolysis of the C-3 ketal and dehydration of the con-
sequent β-hydroxy ketone yields the enone 3. If the C-17
silyl group is lost under these hydrolysis conditions the
C-17 hydroxyl group will be reprotected. Reduction of
10 6β-ethynyl-17β-dimethyl-t-butylsilyloxyandrost-4-en-3-
one, 3, with excess lithium in ammonia followed by rapid
quenching with ammonium chloride affords primarily the
3-keto-4,5α-dihydro compound. (A. Bowers, H.J. Ringold,
and E. Denot, J. Amer. Chem. Soc. 1958, 80, 6115).
15 Sodium borohydride reduction of the C-3 ketone yields
6β-ethynyl-5α-androstan-3β,17β-diol 17-dimethyl-t-butyl-
silyl ether, 4. Protection of the C-3 alcohol as an
acetate and deprotection at C-17 with fluoride ion
yields 6β-ethynyl-5α-androstan-3β,17β-diol 3-acetate, 5.
20 Oxidation of the C-17 hydroxyl group with chromium tri-
oxide/pyridine followed by deprotection of the C-3-
hydroxyl group yields 6β-ethynyl-5α-androstan-3β-ol-17-
one, 6. Higher homologues can be synthesized from 6
by first protecting the ketone and alcohol then using
25 the acetylide anion to react with primary alkyl halides.
The C-6 ethenyl derivates (6 R= CH=CH₂) can be prepared
by reduction of the corresponding C-6 ethynyl derivatives.

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1 <u>Alkenylation and Alkynylation at Carbon-7.</u>

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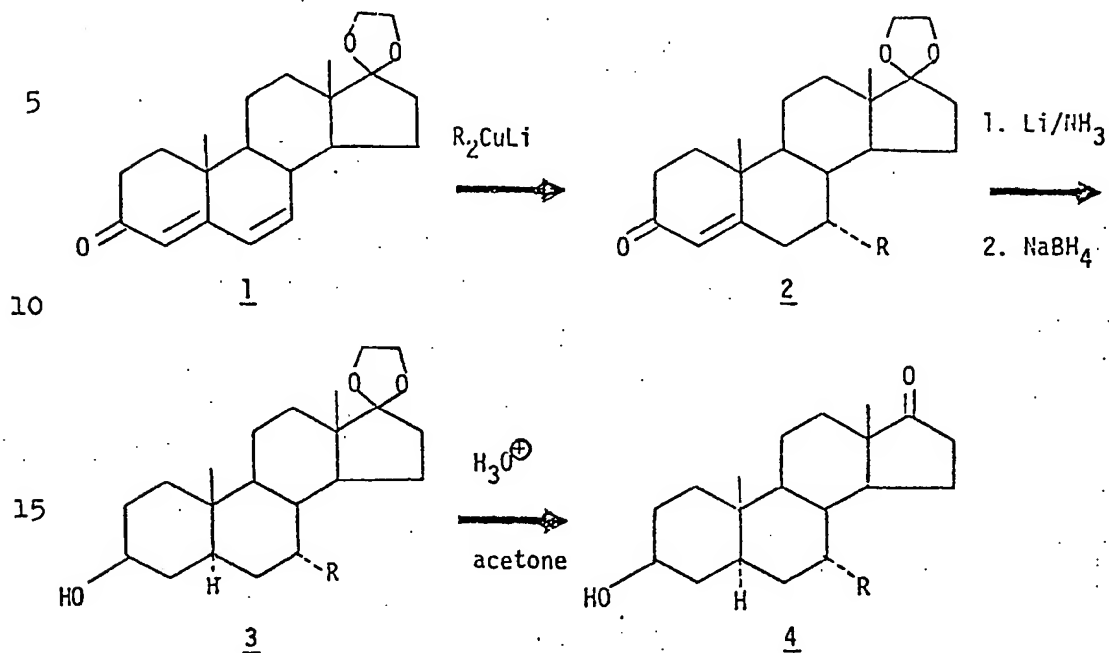
1   Alkenylation of androsta-4,6-dien-3,17-dione 17-ketal $\underline{1}$ with 2-tri-n-butylstannyl ethenyl 1'-pentynyl lithium cuprate ($[C_3H_7C{\equiv}C\text{-}Cu\text{-}CH{=}CHSn\ nBu_3]Li$) yields the 7α-alkenyl steroid $\underline{2}$ (R = $CH{=}CHSn\ nBu_3$). Oxidation using 5 lead tetraacetate proceeds with the loss of tin affords the corresponding acetylene $\underline{2}$)R = $C{\equiv}CH$). Reduction of 7α-ethynylandrost-4-en-3,17-dione 17-ketal $\underline{2}$, (R = $C{\equiv}CH$), with excess lithium in ammonia followed by rapid quenching with ammonium chloride affords primarily the 3-keto-4,5α-10 dihydro compound. Sodium borohydride reduction of the C-3 ketone yields 7α-ethynyl-5α-androstan-3β-ol-17-one 17-ketal $\underline{3}$. Careful acid hydrolysis of the C-17 ketal yields 7α-ethynyl-5α-androstan-3β-ol-17-one $\underline{4}$ (R = $C{\equiv}CH$). Higher homologues can be synthesized from $\underline{4}$, by first protecting 15 the ketone and alcohol, then using the acetylide anion to react with primary alkyl halides. The C-7 ethenyl derivatives $\underline{4}$ (R = $CH{=}CH_2$) can be prepared by reduction of the corresponding C-7 ethynyl derivatives.

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1 <u>Alkenylation and Alkynylation at Carbon-11.</u>
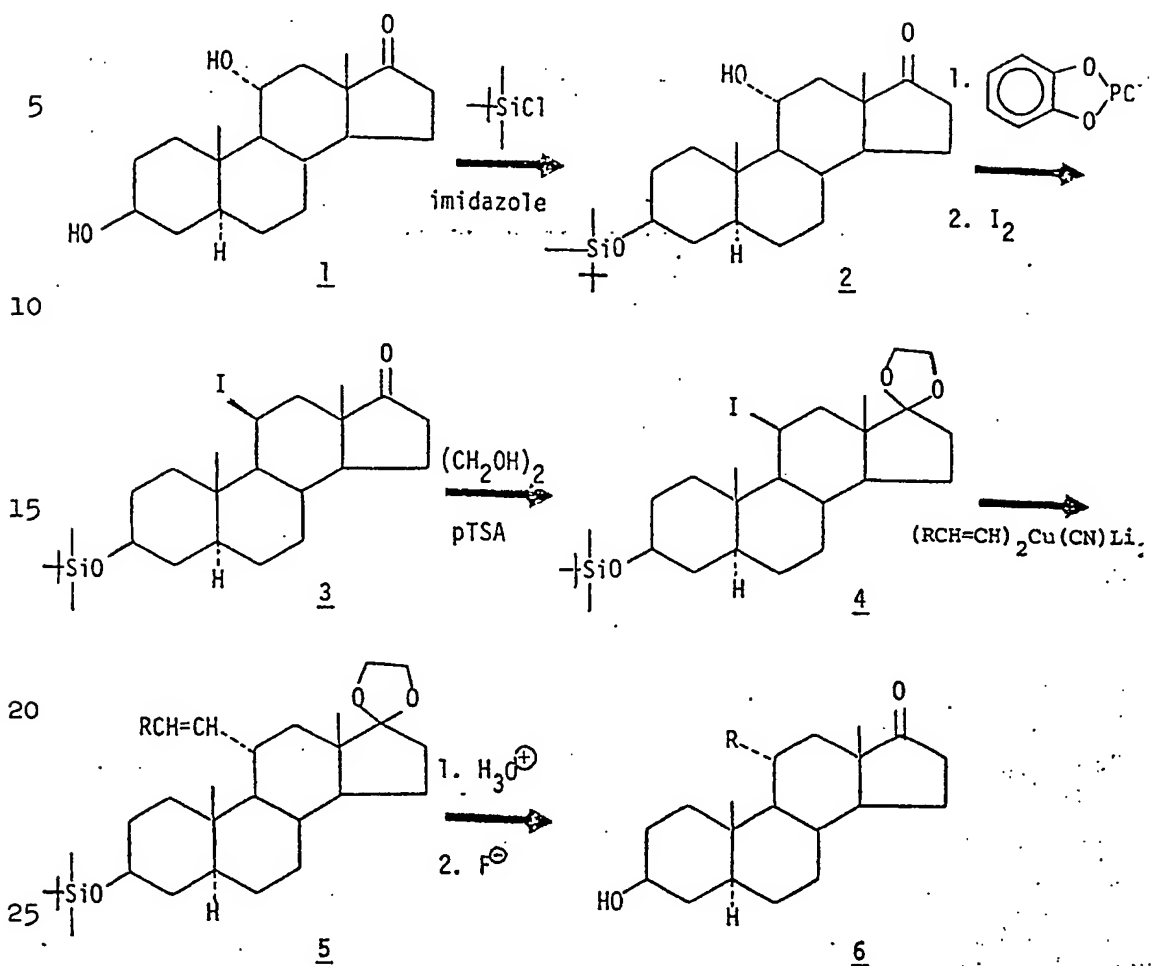
1   Reaction of the less hindered 3β-hydroxy-5α-androstan-17-one <u>1</u> with t-butyldimethylsilyl chloride yields the 3β-t-butyldimethylsilyl ether <u>2</u>. Treatment of this first with catechol phosphochloridate followed

5 by displacement with iodine yields 3β-hydroxy-11β-iodo-5α-androstan-17-one 3-dimethyl-t-butylsilyl ether <u>3</u>. Protection of the C-17 ketone as the 1,3-dioxolane <u>4</u> followed by alkenylation using dialkenyl dilithio cyano cuprate, $(RCH=CH)_2Cu(CN)Li_2$ yields 11α-alkenyl-3β-hydroxy-

10 5α-androstan-17-one-3-t-butyldimethylsilyl ether <u>5</u>. Deprotection of the C-17 ketone and 3β alcohol affords 11α-alkenyl 3β-hydroxy-5α-androstan-17-one <u>6</u>. If <u>6</u> has R=2'-tri-n-butylstannyl ethenyl, then lead tetraacetate oxidation affords 11α-alkynyl-3β-hydroxy-5α-androstan-17-

15 one. <u>6</u>, (R= C≡CH).

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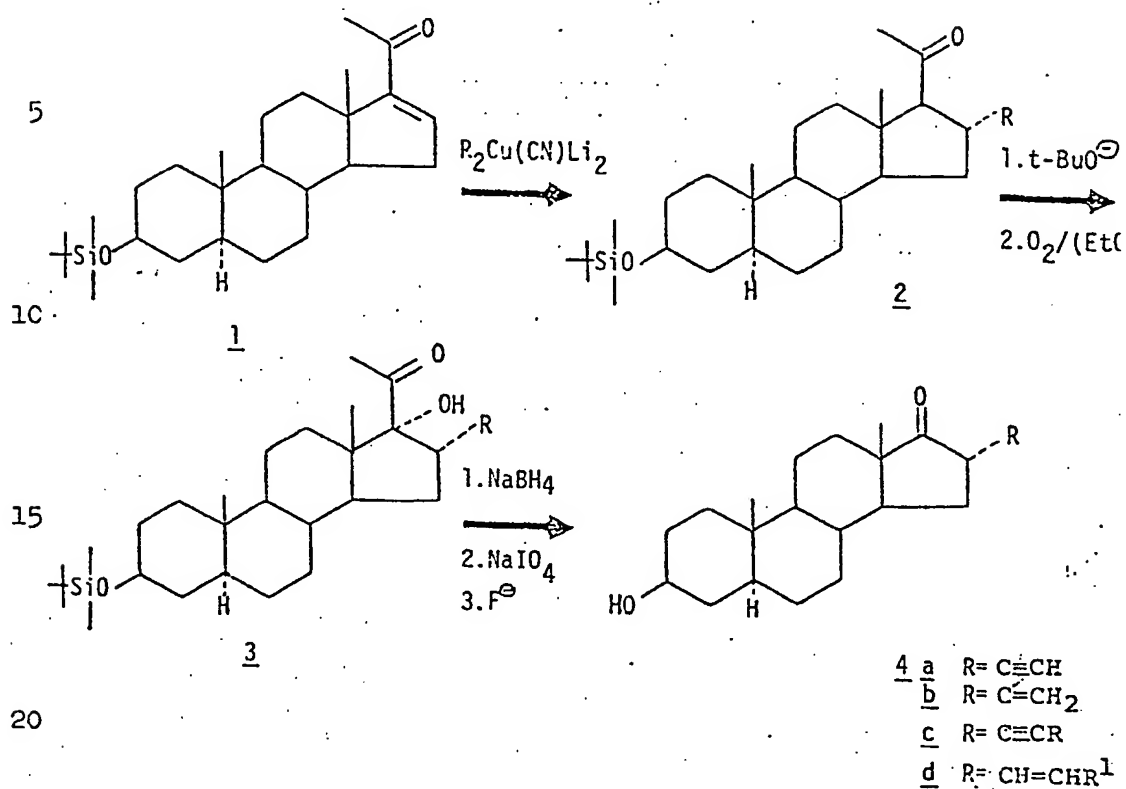
1

Alkenylation and Alkynylation at Carbon-16.

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1C

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$$\underline{4}\ \underline{a}\quad R= C\equiv CH$$
$$\underline{b}\quad R= C=CH_2$$
$$\underline{c}\quad R= C\equiv CR$$
$$\underline{d}\quad R= CH=CHR^1$$

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1   Michael addition of a suitably substituted
organo copper reagent such as 2-tri-n-butylstannyl
ethenyl 1'-pentynyl lithium cuprate ($[C_3H_7C\equiv C-Cu=CH=CH$
Sn $nBu_3]Li$) to 3β-hydroxy-5α-pregna-16-en-20-one 3-t-
5 butyl dimethylsilyl ether 1 yields a 16α-tri-n-butyl-
stannyl ethylene (2, R= $CH=CHSn$ nBu_3). Lead tetra-
acetate oxidation proceeds with the loss of tin and
yields the corresponding acetylide. Treatment of 2
with t-butoxide followed by oxygen generates a C-16α-
10 hydroperoxide which is reduced by triethylphosphite
to 16α-ethynyl-3β,17α-dihydroxy-5α-pregnan-20-one 3-t-
butyldimethylsilyl ether 3. Reduction of the C-20
ketone to an alcohol followed by cleavage of the diol
with sodium periodate and deprotection of the 3β-hydroxyl
15 group with fluoride, yields 16α-ethynyl-3β-hydroxy-5α-
androstan-17-one, 4a (R = $C\equiv CH$). Careful reduction of
the acetylene in 4a should afford the 16α-vinyl sub-
stituted steroids, 4b (R = $CH=CH_2$). Higher homologues
of these substituents may be synthesized via acetylide
20 chemistry using 4a with its hydroxyl and ketone groups
first protected. Reduction of the substituted acetylene
will afford both E and Z olefinic substituents at C-16.
       In order to determine the pharmacological
activity of the novel and other-steroids of the present
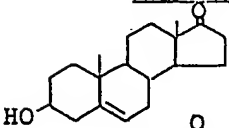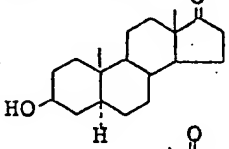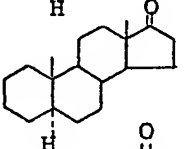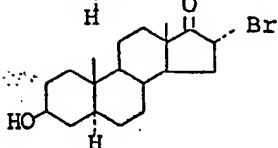25 invention, the following experiments are carried out.
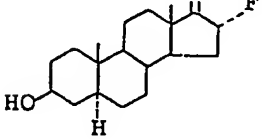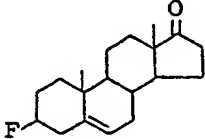

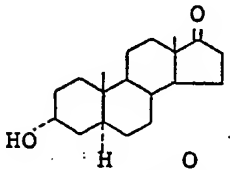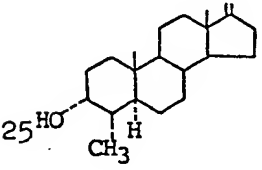30

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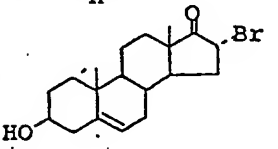
1 <u>Inhibition of G6PDH</u>
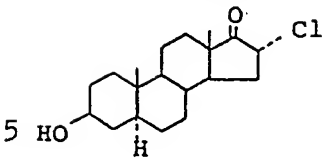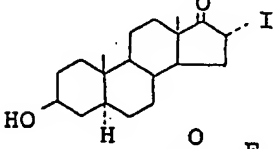
The compounds are screened as inhibitors
of purified bovine adrenal G6PDH activity as one pre-
5 dictor of cancer preventive action. The results are
shown in Table I:

<u>Table I</u>

Compound	No.	Conc.	Per Cent Inhibition
	<u>1</u>	10 μm 1 μm 0.1 μm	53 36 12
	<u>2</u>	10 μm 1 μm 0.1 μm	82 64 36
	<u>3</u>	10 μm 1 μm 0.1 μm	80 69 10
	<u>4</u>	10 μm	90
	<u>5</u>	10 μm	74

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	Compound	No.	Conc.	Per Cent Inhibition
5		6	10 µm	72
		7	10 µm	51
10		8	10 µm	66
15		9	10 µm	22
20		10	10 µm	35
25		11	10 µm	35

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	Compound	No.	Conc.	Per Cent Inhibition
1		<u>12</u>	10 µm	48
5				
		<u>13</u>	10 µm 1 µm 0.1 µm	73 55 58
10				
		<u>14</u>	10 µm 1 µm 0.1 µm	60 15 0
15		<u>15</u>	10 µm 1 µm 0.1 µm	46 40 26
20		<u>16</u>	10 µm 1 µm 0.1 µm	53 40 25
25		<u>17</u>	10 µm 1 µm 0.1 µm	60 45 49
30		<u>18</u>	10 µm 1 µm 0.1 µm	45 18 10

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1   <u>Inhibition of TPA (Tumor Promoter) Stimulation</u>
<u>of Mouse Epidermal DNA</u>

<u>Synthesis rate by orally administered steroids.</u>

The inhibition of tumor promoter stimulation of
5 mouse epidermal DNA synthesis rate by steroids may also
contribute to cancer preventive activity. The following
assay is used:

ICR male mice (7-9 weeks old) were shaved on
the back 1-2 days before use. Only mice showing no hair
10 regrowth were used. Animals were orally intubated with
a particular steroid suspended by homogenization in
sesame oil or with sesame oil alone (controls). One
hour later TPA (10 µg in 0.2 ml of acetone) or acetone
vehicle was applied topically to the shaved skin. Twenty
15 hours later, mice were injected i.p. with 60 µCi of ^{3}H-
thymidine 20 minutes before sacrifice. The animals were
killed by cervical dislocation and the residual hair
was removed with a depilatory agent. Epidermal scrapings
were prepared according to the procedures of Hennings
20 et al. (Cancer Res. <u>28</u>, 53, 1968), homogenized in dis-
tilled water at 4°C, and the macromolecules precipitated
with 0.4 N trichloroacetic acid (TCA). Following 6
washes with absolute ethanol at room temperature, the
nucleic acids were hydrolyzed with 0.5 N TCA at 70°C for
25 5 minutes. The hydrolysate (0.2 ml aliquots) were counted
in an Intertechnique scintillation counter and assayed
for DNA by the diphenylamine reaction.

The data are expressed as counts per minute
(cpm) of tritium per µg of DNA.
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	cpm/ug DNA
1 Control (no TPA and no steroid)	37 ± 6.1 (number (n) of animals = 3)
TPA	101 ± 20
TPA + DHEA (compound 1)* (400 mg/kg)	42 ± 7
5 TPA + DHEA (200 mg/kg)	88 ± 9.3
TPA + DHEA (100 mg/kg)	87 ± 8.2
TPA + Compound 2* (200 mg/kg)	100 ± 6.0
TPA + Compound 2* (100 mg/kg)	97 ± 15
TPA + Compound 3* (200 mg/kg)	64 ± 10
10 TPA + Compound 3* (100 mg/kg)	113 ± 21

*of Table 1

Conclusion: DHEA is active at blocking the TPA stimu-
lation in DNA synthesis rate at 400 mg/kg but not at
15 200 mg/kg or 100 mg/kg. Compound 2 is not active at
200 mg/kg or 100 mg/kg. Other tests in which compound 2
or compound 3 and DHEA were given by i.p. injection in
a dose-response experiment indicated that 2 is about
20 as active as DHEA and compound 3 was more active in
blocking the TPA stimulation in DNA synthesis rate.
Compound 3 appears somewhat more active than
DHEA at the dose of 200 mg/kg administered orally.

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1 <u>Compound 2 or 3 in comparison with DHEA by Intraperi-
toneal Injection.</u>

Steroids were suspended in sterile 95% saline –
5% Emulphor and injected intraperitoneally. Otherwise
5 conditions were the same as when steroids were orally
administered.

Compound 2 vs. DHEA	cpm/µg DNA
10 Control (no steroid or TPA)	63 ± 3.9 (n = 2)
TPA	170 ± 2.2
DHEA (10 mg/kg i.p.) + TPA	66 ± 2.1
DHEA (2 mg/kg i.p.) + TPA	105 ± 12
DHEA (0.4 mg/kg i.p.) + TPA	157 ± 4.2
15 Cpd 2 (10 mg/kg i.p.) + TPA	58 ± 0.9
Cpd 2 (2 mg/kg i.p.) + TPA	94 ± 1.8
Cpd 2 (0.4 mg/kg i.p.) + TPA	148 ± 3.0

Compound 3 vs. DHEA	cpm/µg DNA
20 Control (no steroid or TPA)	46 ± 5.3
TPA	114 ± 37
Cpd 3 (10 mg/kg i.p.) + TPA	8.9 ± 3.0
Cpd 3 (2 mg/kg i.p.) + TPA	27 ± 8.8
Cpd 3 (0.4 mg/kg i.p.) + TPA	32 ± 2.4

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1 Compound 16* vs. DHEA

A similar oral dose-response experiment with compound 16 and DHEA was performed.

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	cpm/µg DNA
Control (no TPA, no steroid)	55 ± 3.7 (n=2)
TPA	162 ± 2.1
TPA + DHEA (400 mg/kg)	50 ± 2.8
TPA + DHEA (200 mg/kg)	155 ± 1.6
10 TPA + DHEA (100 mg/kg)	169 ± 11
TPA + Compound 16* (400 mg/kg)	39 ± 1.1
TPA + Compound 16* (200 mg/kg)	44 ± 2.5
TPA + Compound 16* (100 mg/kg)	100 ± 19

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*of Table I

Conclusion: Compound 16 is about 3X as active as DHEA in this test.

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1 Anti-Obesity Test

Male A/J mice (5 weeks old) were obtained
from the Jackson Laboratory and were housed in poly-
carbonate cages (5 mice/cage) in animal quarters main-
5 tained at $24 \pm 1°C$ with 12 hours of light and 12 hours
of darkness each day. One week after arrival, the
mice were placed on a chow diet containing varying con-
centrations of DHEA or other steroid. Animals were
weighed weekly; food consumption was determined weekly
10 by subtracting the amount of food remaining in the
cage from the amount added.

Compound 3 vs. DHEA

15 Week	Control (no steroid)	DHEA 0.7%	Cpd 3 0.35% mean weekly weight in grams (n=5)	Cpd 3 0.7%
0	21.6 ± 2.6	21.1 ± 2.8	21.8 ± 2.2	21.6 ± 2.6
1	22.6 ± 2.1	15.2 ± 1.8	22.6 ± 1.8	20.0 ± 1.9
2	23.4 ± 1.8	17.0 ± 2.0	23.6 ± 1.8	21.3 ± 1.8
20 3	24.6 ± 2.3	17.8 ± 1.1	24.8 ± 1.8	21.8 ± 1.6
4	25.4 ± 2.5	18.8 ± 1.1	24.6 ± 1.8	22.0 ± 16
5	26.0 ± 2.3	18.2 ± 1.3	24.8 ± 1.9	21.4 ± 1.1

There was an initial depression in food consump-
25 tion in the DHEA treated mice in the first week. There-
after the food consumption was equal to or slightly
greater than the control mice.

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1 Compound 2 vs. DHEA

Week	Control	DHEA 0.35%	DHEA 0.7%	Cpd 2 0.35%	Cpd 2 0.7%
		mean weekly weight in grams (n=5)			
0	21.6 ± 1.3	21.8 ± 2.2	22.0 ± 2.5	21.8 ± 1.6	21.3 ± 1.8
1	21.4 ± 2.1	20.0 ± 2.3	17.0 ± 1.9	21.8 ± 1.3	20.6 ± 2.3
2	22.0 ± 0.7	19.2 ± 1.8	17.8 ± 1.8	22.0 ± 1.2	20.6 ± 0.5
3	22.2 ± 0.8	19.6 ± 2.8	18.4 ± 1.8	22.8 ± 1.3	20.8 ± 0.8
4	24.0 ± 1.0	22.2 ± 2.4	19.2 ± 1.6	24.2 ± 1.1	22.6 ± 0.6
5	24.8 ± 0.8	21.8 ± 2.2	20.2 ± 1.9	24.4 ± 1.7	22.4 ± 0.5
6	25.2 ± 1.1	22.8 ± 2.3	19.8 ± 2.0	24.8 ± 1.5	23.5 ± 0.9
7	25.6 ± 1.1	23.2 ± 2.4	20.6 ± 2.2	25.4 ± 1.7	23.8 ± 0.8

Compound 16 vs. DHEA

Week	Control	DHEA 0.18%	DHEA 0.35%	Cpd 16 0.18%	Cpd 16 0.35%
		mean weekly weight in grams (n=5)			
0	21.6 ± 2.8	22.4 ± 1.8	22.4 ± 2.3	22.6 ± 2.7	22.0 ± 2.5
1	23.0 ± 1.6	20.4 ± 1.9	16.0 ± 1.6	19.8 ± 2.3	16.8 ± 1.6
2	24.4 ± 1.6	21.1 ± 0.5	20.2 ± 0.9	19.4 ± 2.1	18.2 ± 0.8
3	25.6 ± 1.9	22.4 ± 0.8	21.0 ± 0.8	19.4 ± 2.1	17.2 ± 1.7
4	26.6 ± 1.5	23.6 ± 1.5	22.2 ± 0.9	19.6 ± 2.2	17.0 ± 1.2

Compound 16 is more than 2X as active as DHEA in this test.

1 Anti-Hyperglycemic Activity

Coleman et al. (Diabetes 31, 830, 1982) reported
that administration of DHEA (0.4% of the diet) produced
a marked hypoglycemic effect in C57BL/KsJ-db/db mice and
5 significantly prolonged their lifespan. The authors n
noted that "diabetes is more severe and develops more
rapidly in males and can be improved or circumvented by
combined estradiol and progesterone treatment" and sug-
gested that the therapeutic effect of DHEA might result
10 from its metabolism to estrogens. Compound 16 (which
has been found herein to be devoid of estrogenic activity
in the rat uterotrophic test) was tested in this model.

C57BL/KsJ-db/db 8 week old female mice were
obtained and housed in polycarbonate cages
15 in animal quarters maintained at 24°C with 12 h of light
and 12 h of darkness each day.

Mice were placed on a control chow or a chow diet
containing either 0.4% DHEA or 0.2% of compound 16.

For determination of blood glucose levels, mice
20 were bled from the orbital sinus using a heparinized
capillary tube. 0.2 ml of blood was added to 1.8 ml
of water to hemolyze the blood and glucose concentration
was determined using the glucose oxidase assay.

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1   <u>Blood Glucose Levels (mg/deciliter)</u>

Week	Control (n=6)	DHEA (0.4%) (n=6)	Cpd. 16 (0.2%) (n=6)
5 0	232 ± 43	232 ± 28	244 ± 32
1	336 ± 37	132 ± 14	143 ± 21
2	394 ± 20	135 ± 37	134 ± 13

All mice placed
10 on control diet.

24 hrs.	399 ± 15	192 ± 32	147 ± 10
48 hrs.	397 ± 13	310 ± 37	262 ± 23

When mice were taken off diets containing DHEA
15 or compound <u>16</u> and placed on control diet, blood glucose
levels increased at 24 and 48 hrs. but significantly more
slowly in the mice that had received compound <u>16</u>.

<u>Anti-Autoimmune Activity</u>

20   New Zealand Black (NZB) mice develop a pro-
gressive autoimmune, Coomb's positive hemolytic anemia
with age. It has been previously found that long-term
treatment of NZB mice with DHEA significantly inhibits
the rate of development of the autoimmune anemia. In
25 other studies reported herein, we have determined that
certain steroids, such as compound <u>16</u>, have retained
the anti-obesity, cancer preventive, and anti-hyper-
glycemic action of DHEA without any apparent estrogenic
effect. There is a reasonable probability that such
30 steroids will also retain the anti-autoimmune activity
of DHEA.

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The following are additional data on the anti-hyperglycemic effect of compound 16 vs. DHEA.

Blood Glucose Levels (mg/deciliter)

Week	Control (n=7)	DHEA (0.4%) (n=7)	Cpd 16(0.2%) (n=7)
0	213 ± 54	214 ± 59	216 ± 61
1	292 ± 29	161 ± 19	135 ± 15
2	335 ± 20	145 ± 19	118 ± 12
3	354 ± 27	117 ± 12	102 ± 8
4	387 ± 15	112 ± 4	105 ± 5

Compound 16 is more effective in lowering blood glucose concentration at an administered dose of 0.2% in the diet than is DHEA at a dose of 0.4%.

Anti-Hypercholesterolemic Activity

Six-week old female ICR mice were obtained and placed in animal quarters at 24°C with 5 animals/cage with food and water ad libitum. All mice (except the control group) received 0.1% PTU (propylthiouracil) in their drinking water. Mice receiving DHEA or compound 4 were injected with the steroid i.p. (15 mg/kg) 3X weekly.

For serum cholesterol determinations, mice were bled from the orbital sinus. Blood was allowed to coagulate and was centrifuged to obtain serum. Cholesterol was measured according to the procedure of Rao et al. (Lipids 12, 1078, 1977).

Experimental Group	No. Mice	Serum Cholesterol (mg%)		
		before treatment	1 wk after treatment	2 wk after treatment
Control (no steroid or PTU)	30	53.8 ± 9.3	54.0 ± 4.0	59.2 ± 7.2
PTU	40	51.3 ± 6.1	75.6 ± 12.9	76.6 ± 2.4
PTU + DHEA	10	61.0 ± 6.8	57.3 ± 3.6	57.5 ± 3.2
PTU + Cpd 4	10	58.1 ± 5.6	53.4 ± 5.2	———

Uterotrophic Test for Estrogenic Activity. Compound 16 vs. DHEA and Estradiol-benzoate. Compounds Given by Oral Administration.

Twenty-two day old rats were obtained from Charles River Laboratories. Animals were used at 29 days of age. Test steroids were suspended by homogenization in sesame oil. Rats were orally intubated at 1-2 P.M. for 3 days with a test steroid in sesame oil or with sesame oil alone (control). On the 4th day the animals were killed and the uteri were removed and weighed.

	Mean Uterine Weight (Mgs) (n=6)
Control (no steroid)	166 ± 34
DHEA (400 mg/kg)	261 ± 23 (p<0.001, greater than control)
Compound 16 (400 mg/kg)	174 ± 20
Compound 16 (200 mg/kg)	189 ± 14
Estradiol-benzoate (14 µg/kg)	244 ± 44 (p<0.01)

1 Compound 2 vs. DHEA. Compounds Administered by Subcutaneous Injection

Steroids were dissolved in 2 ml ethanol and
5 brought up to 5 ml volume with propylene glycol. Concentrations were such that 1 µl/gm body weight delivered the indicated dose.

Mean Uterine Weight (Mgs) (n=4 or 5)

10 Control (no steroid)	152 ± 22
DHEA (60 mg/kg) $p < 0.02$	290 ± 72
DHEA (10 mg/kg)	152 ± 17
Compound 2 (60 mg/kg)	127 ± 32
Compound 2 (10 mg/kg)	151 ± 32

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The above test is being repeated at the oral dose of 400 mg/kg, which would have been more appropriate, since this is a therapeutic dose.

Conclusion: Neither compounds 16 nor 2 are estrogenic at
20 doses at which DHEA is significantly estrogenic.

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1 Summary

Compound 16 is without estrogenic activity in the uterotrophic test and is approximately 3X as active as DHEA in the mouse skin tumor promoter assay and in 5 the anti-obesity test. Compound 2 is without estrogenic activity but is about 1/3 as active as compound 16 in the skin tumor promoter test and only 1/6 as active in the anti-obesity test. However, compound 3 is only about 1/2 as active as compound 16 in the skin tumor 10 promoter test and 1/6 as active in the anti-obesity test.

Compound 15 is about as active as DHEA and compound 16 in the G6PDH inhibition test.

The compounds, i.e. therapeutic agents of this 15 invention may be administered alone or in combination with pharmaceutically-acceptable carriers, the proportion of which is determined by the solubility and chemical nature of the compound, chosen route of administration and standard pharmaceutical practice. For example, they may 20 be administered orally in the form of tablets, pills or capsules containing such excipients as starch, milk sugar, certain types of clay and so forth. They may be administered orally in the form of solutions which may contain coloring and flavoring agents or they may be injected 25 parenterally, that is, intramuscularly, intravenously or subcutaneously. For parenteral administration, they may be used in the form of a sterile solution containing other solutes, for example, enough saline or glucose to make the solution isotonic.
30 The physician will determine the dosage of the present therapeutic agents which will be most suitable and it will vary with the form of administration and the

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1 particular compound chosen, and furthermore, it will vary
with the particular patient under treatment. He will
generally wish to initiate treatment with small dosages
substantially less than the optimum dose of the compound
5 and increase the dosage by small increments until the
optimum effect under the circumstances is reached. It
will generally be found that when the composition is
administered orally, larger quantities of the active
agent will be required to produce the same effect as
10 a smaller quantity given parenterally. The compounds
are useful in the same manner as comparable therapeutic
agents and the dosage level is of the same order of
magnitude as is generally employed with these other
therapeutic agents.

15   When given orally, the therapeutic doses of
the compounds of the present invention are generally
in the range of from about 4 to about 450 mg/kg/day
depending upon the particular mammalian host and the
particular effect desired, e.g. cancer preventive, anti-
20 obesity, anti-diabetes, etc., when given by parenterally,
the compounds are administered generally in dosages of,
for example, 0.5 to about 15 mg/kg/day also depending
upon the host and effect desired.

       Obviously, other modifications and variations
25 of the present invention are possible in the light of
the above teachings. It is, therefore, to be understood
that changes may be made in the particular embodiments
of this invention which are within the full intended scope
of the invention as defined by the appended claims.

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CLAIMS

1. A compound of the formula
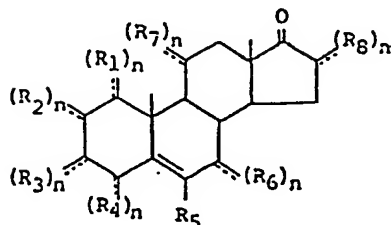
wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 and R_8 are each independently selected from the group consisting of hydrogen, alkyl, alkenyl, alkynyl, halogen and hydroxyl, n is an integer from 1 to 2 inclusive with the proviso that when R_1, R_2, R_3, R_4, R_5, R_6, R_7 or R_8 is alkenyl or alkynyl, n is 1; and with the further provisos that when R_3 is hydroxy, any one of the substituents R_1, R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_1 may only be alkyl when any one of R_2, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_2 may only be alkyl when any one of R_1, R_4, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_4 may only be halogen when R_1, R_2, R_5, R_6, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_6 may only be hydroxy or methyl when R_1, R_2, R_4, R_5, R_7 or R_8 is other than hydrogen; when R_3 is hydroxy, R_7 may only be hydroxy when R_1, R_2, R_4, R_5, R_6 or R_8 is other than hydrogen; and when R_3 is hydroxy, R_8 may only be methyl, ethyl, hydroxy or halogen when R_1, R_2, R_4, R_5, R_6 or R_7 is other than hydrogen; and when R_3 is hydroxy, R_5 may be alkyl only when R_1, R_2, R_4, R_6, R_7 or R_8 are other than hydrogen.

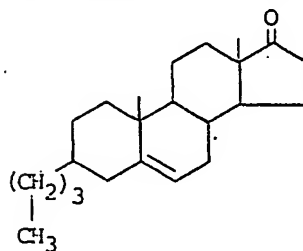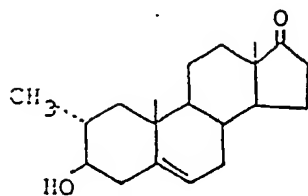1   9.   The compound as in Claim 1 of the formula
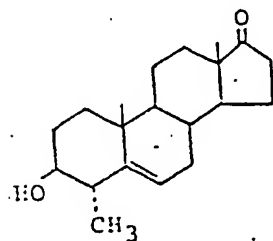
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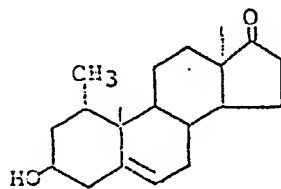   10. The compound as in Claim 1 of the formula

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           11. The compound as in Claim 1 of the formula

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1   12. The compound as in Claim 1 which is 3-β, 16α-dimethyl androst-5-en-17-one.

     13. The compound as in Claim 1 which is 3-β-methyl-16α-hydroxy-androst-5-en-17-one.

5   14. A therapeutic composition comprising a compound as in any of Claims 1-13 and a carrier therefor.

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